

=> d his

(FILE 'HOME' ENTERED AT 06:51:51 ON 21 APR 2005)  
DEL HIS

FILE 'HCAPLUS' ENTERED AT 06:53:57 ON 21 APR 2005

L1 712 S ?PROUK? OR ?PROUROKINASE? OR PRO() (UK OR UROKINASE)  
L2 3 S RPRO() (UK OR UROKINASE)  
L3 712 S L1,L2

FILE 'REGISTRY' ENTERED AT 06:55:45 ON 21 APR 2005

L4 1 S 82657-92-9  
E PROUROKINASE  
L5 151 S E3

FILE 'HCAPLUS' ENTERED AT 06:57:31 ON 21 APR 2005

L6 684 S L4  
L7 714 S L5  
L8 545 S ABT 187 OR ABT187 OR PRO U PA OR PROLYSE OR PROLYZE OR PUK OR  
L9 1171 S L3,L6-L8  
L10 7 S PET29A  
L11 20 S ?PET29?  
L12 4 S L9 AND L10,L11  
L13 9 S L9 AND T7  
L14 6 S L9 AND (SHINE OR DALGARNO)  
L15 16 S L9 AND (BL21 OR DE3 OR RIL)  
L16 7 S L12-L14 AND L15  
E E COLI/CT  
E ESCHERICHIA/CT  
L17 148991 S E3+OLD,NT,PFT,RT OR E14+OLD,NT,PFT,RT  
L18 248421 S ("E" OR ESCHERICH?) () COLI  
L19 176 S L9 AND L17,L18  
L20 2 S L19 AND TYPE B  
L21 108 S L9 AND B  
L22 3 S L20,L21 AND L10-L16  
L23 153 S L9 AND ESCHERI?  
L24 21 S L23 AND L10-L16  
L25 3 S L24 AND (TYPE B OR B)  
L26 27 S L12-L16,L20,L22,L25  
L27 235 S L19,L21,L23,L24 NOT L26  
L28 4 S L26 AND ?MUTANT?  
L29 2 S L26 AND ?MUTAT?  
L30 36 S L27 AND (?MUTANT? OR MUTAT?)  
L31 2 S L26 AND ?MUTAGEN?  
L32 15 S L27 AND ?MUTAGEN?  
L33 4 S L28,L29,L31  
L34 41 S L30,L32 NOT L33  
L35 2 S L9 AND FLEX?(L) LOOP?  
L36 1 S L9 AND WOBBL?(L) LOOP?  
L37 2 S L35,L36  
L38 6 S L9 AND (LYS300 OR LYS 300)  
L39 0 S L9 AND (LYSINE300 OR LYSINE 300)  
L40 27 S L9 AND (HIS OR HISTID?)  
L41 124 S L9 AND (LYS OR LYSINE OR LYSYL?)  
L42 12 S L40 AND L41

FILE 'REGISTRY' ENTERED AT 07:06:40 ON 21 APR 2005

L43 1 S 56-87-1  
L44 3 S (D-LYSINE OR DL-LYSINE)/CN OR L43  
L45 3 S (L-HISTIDINE OR D-HISTIDINE OR DL-HISTIDINE)/CN

FILE 'HCAPLUS' ENTERED AT 07:07:20 ON 21 APR 2005

L46 24 S L44 AND L9

L47 6 S L45 AND L9  
 L48 13 S L38,L41,L46 AND L40,L47  
 L49 2 S L48 AND L10-L16,L26  
 L50 4 S L48 AND L27  
 L51 3 S L48 AND L28-L37  
 L52 6 S L49-L51  
 L53 5 S L52 NOT CRYOGEL/TI  
 L54 96 S L10-L16,L20,L22,L25,L26,L28-L37,L48  
 L55 5 S L54 AND L53  
 L56 4 S L55 NOT MMP 3/TI  
 L57 91 S L54 NOT L55  
 L58 15 S L57 AND PROUROKINASE/TI  
 L59 19 S L56,L58  
 L60 76 S L57 NOT L59  
 L61 35 S L60 AND L6  
 L62 38 S L60 AND L7  
 L63 57 S L61,L62,L59  
 L64 38 S L54 NOT L55-L56,L58,L59,L61-L63  
 SEL DN AN 13-16 22 24 26 29-34 36-38  
 L65 16 S L64 AND E1-E48  
 L66 73 S L63,L65  
 L67 72 S L66 AND ?UROKINASE?  
 L68 1 S L66 NOT L67  
 E SARMIENTOS P/AU  
 L69 46 S E3,E4  
 E PAGANI M/AU  
 L70 45 S E3-E8,E18  
 L71 9 S L69,L70 AND L9  
 L72 12 S L69,L70 AND ?UROKINASE?  
 L73 12 S L71,L72  
 L74 2 S US20050019863/PN OR (WO2004-US11840 OR US2004-826598# OR US20  
 L75 1 S L74 AND L9  
 L76 1 S L74 AND ?UROKINASE?  
 L77 12 S L75,L76,L73  
 L78 78 S L77,L67  
 L79 78 S L78 AND L1-L3,L6-L42,L46-L78  
 L80 75 S L79 AND (PD<=20030418 OR PRD<=20030418 OR AD<=20030418)  
 L81 3 S L79 NOT L80  
 L82 78 S L79-L80  
 L83 49 S L82 AND (?MUTANT? OR ?MUTAGEN? OR ?MUTAT?)  
 E MUTANT/CT  
 E MUTAT/CT  
 L84 332100 S E7+OLD,NT,PFT,RT  
 E MUTAGEN/CT  
 L85 202397 S E5+OLD,NT,PFT,RT OR E5-E10  
 L86 268465 S E16+OLD,NT,PFT,RT  
 L87 13 S L82 AND L84-L86  
 L88 50 S L83,L87  
 L89 45 S L82 AND (RECOMBIN? OR CHIMER?)  
 E RECOMBINANT/CT  
 L90 0 S L82 AND E11+OLD,NT,PFT,RT  
 L91 0 S L82 AND E41+OLD,NT,PFT,RT  
 L92 28 S L82 AND E48+OLD,NT,PFT,RT  
 E E48+ALL  
 L93 28 S L82 AND E9+OLD,NT,PFT,RT  
 L94 4 S L82 AND E7+OLD,NT,PFT,RT  
 L95 70 S L88-L94  
 L96 8 S L82 NOT L95  
 SEL DN AN 1 4 5  
 L97 5 S L96 NOT E1-E9  
 L98 75 S L95,L97

=&gt; fil reg

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STRUCTURE FILE UPDATES: 20 APR 2005 HIGHEST RN 848887-73-0  
DICTIONARY FILE UPDATES: 20 APR 2005 HIGHEST RN 848887-73-0

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\* effective March 20, 2005. A new display format, IDERL, is now \*  
\* available and contains the CA role and document type information. \*  
\*  
\*\*\*\*\*

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information enter HELP PROP at an arrow prompt in the file or refer  
to the file summary sheet on the web at:  
<http://www.cas.org/ONLINE/DBSS/registryss.html>

=> d ide can l4

L4 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2005 ACS on STN  
RN 82657-92-9 REGISTRY  
ED Entered STN: 16 Nov 1984  
CN Kinase (enzyme-activating), prouro- (9CI) (CA INDEX NAME)  
OTHER NAMES:  
CN ABT-187  
CN Pro u-PA  
CN Pro-UK  
CN Pro-urokinase-type plasminogen activator  
CN Prolyse  
CN Prourokinase  
CN Prourokinase plasminogen activator  
CN PUK  
CN Scu-PA  
CN Single-chain pro-urokinase  
CN Single-chain urokinase  
CN Single-chain urokinase-type plasminogen activator  
CN Thombolyse  
CN Tomieze  
MF Unspecified  
CI COM, MAN  
LC STN Files: ADISINSIGHT, ADISNEWS, AGRICOLA, BIOBUSINESS, BIOSIS,  
BIOTECHNO, CA, CAPLUS, CBNB, CIN, CSCHM, DDFU, DRUGU, EMBASE, IPA,  
MRCK\*, PHAR, PROMT, PROUSDDR, TOXCENTER, USPAT2, USPATFULL  
(\*File contains numerically searchable property data)

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*

682 REFERENCES IN FILE CA (1907 TO DATE)  
70 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA  
684 REFERENCES IN FILE CAPLUS (1907 TO DATE)

REFERENCE 1: 142:316831  
REFERENCE 2: 142:293700  
REFERENCE 3: 142:293158  
REFERENCE 4: 142:285326  
REFERENCE 5: 142:255442  
REFERENCE 6: 142:237004  
REFERENCE 7: 142:211364  
REFERENCE 8: 142:109438  
REFERENCE 9: 142:11628  
REFERENCE 10: 141:423372

=> fil hcaplus

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FILE COVERS 1907 - 21 Apr 2005 VOL 142 ISS 17  
FILE LAST UPDATED: 20 Apr 2005 (20050420/ED)

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This file contains CAS Registry Numbers for easy and accurate substance identification.

=> d l98 bib abs hitrn retable tot

L98 ANSWER 1 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 2004:936297 HCAPLUS  
DN 142:255442  
TI Construction, expression, and characterization of a recombinant annexin B1-low molecular weight urokinase chimera in *Escherichia coli*  
AU Yan, Hong-Li; Wang, Wei-Ting; He, Yan; Zhao, Zhuan-You; Gao, Yuan-Jian; Zhang, Yi; Sun, Shu-Han  
CS Department of Medical Genetics, Second Military Medical University, Shanghai, 200433, Peop. Rep. China  
SO Acta Biochimica et Biophysica Sinica (2004), 36(3), 184-190

*Wang*

CODEN: ABBSC2; ISSN: 1672-9145

PB Shanghai Scientific and Technical Publishers

DT Journal

LA English

AB To produce a thrombi-targeting plasminogen activator, low mol. weight **single-chain urokinase** gene (scuPA32k) was spliced with the full-length cDNA of annexin B1 gene (anxB1) by overlap extension method. The fused gene anxB1scuPA was ligated into pET28a vector, transformed into *E. coli* BL21-RIL, and then induced to express under the control of T7 promoter. The AnxB1 ScuPA protein expressed amounted to 22% of the total bacterial proteins. The product was refolded, and then purified by using DEAE Sepharose fast flow ion-exchange column and Superdex S-200 gel-filtration column. HPLC anal. revealed that the final purity is about 95%. The specific activity of AnxB1ScuPA, measured as amidolytic activity, reached 100,000 IU/mg. It had a similar S2444 catalytic efficiency (kcat/Km) to ScuPA32k, and also showed high activated-platelet membrane-binding activity and anticoagulant activity, indicating that the **chimera** fully retained the components of enzymic and membrane-binding activities of the parent mols. In vivo test revealed that, the dogs administered with AnxB1ScuPA had less reperfusion time, higher reperfusion ratio, and less bleeding effects than those with **urokinase**. These findings indicated that AnxB1ScuPA might have advantages over current available thrombolytic agents.

IT 82657-92-9, **Single-chain urokinase**

RL: BSU (Biological study, unclassified); BIOL (Biological study) (construction, expression, and characterization of a **recombinant annexin B1-low mol. weight urokinase chimera** in *Escherichia coli*)

## RETABLE

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	Referenced File
Bradford, M	1976	72	248	Anal Biochem	HCAPLUS
Cahill, M	1993	50	221	Br J Biomed Sci	HCAPLUS
Deckmyn, H	1994	87	562	Br J Haematol	HCAPLUS
Ho, S	1989	77	51	Gene	HCAPLUS
Laemmli, U	1970	227	680	Nature	
Lenich, C	1992	68	539	Thromb Haemost	HCAPLUS
Lu, S	1999		380	Current Protocols fo	
Marshak, D	1996		12	Strategies for Prote	
Sambrook, J	2001		66	Molecular Cloning:A	
Tanaka, K	1996	35	922	Biochemistry	HCAPLUS
Vrkljan, M	1994	11	1004	Pharm Res	HCAPLUS
Yan, H	2002	119	1	Mol Biochem Parasito	HCAPLUS
Yan, H	2004	25	54	Science in China	
Zhang, Y	2002	23	378	Science in China	
Zhao, Z	2001	37	420	Journal of Nanjing U	HCAPLUS

L98 ANSWER 2 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2004:927004 HCAPLUS

DN 141:378919

TI Methods, devices, and compositions for lysis of occlusive blood clots while sparing wound-sealing clots

IN Gurewich, Victor; Williams, John N.; Liu, Jian-Ning; **Sarmientos, Paolo; Pagani, Massimiliano**

PA Thrombolytic Science, Inc., USA

SO PCT Int. Appl., 90 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

PATENT NO.

KIND

DATE

APPLICATION NO.

DATE

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PI  WO 2004093797      A2      20041104      WO 2004-US11840      20040416 <--
    W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
      CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
      GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
      LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI,
      NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY,
      TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW,
    RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ,
      BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE,
      ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,
      SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN,
      TD, TG

    CA 2426115      AA      20041018      CA 2003-2426115      20030422 <--
    US 2005019863      A1      20050127      US 2004-826598      20040416 <--
    US 2005031607      A1      20050210      US 2004-826826      20040416 <--
PRAI US 2003-463930P      P      20030418      <--
    US 2003-464002P      P      20030418      <--
    US 2003-464003P      P      20030418      <--
AB  It has now been discovered that various mutant forms of
    pro-urokinase ('pro-UK') perform in
    the manner of pro-UK in lysing 'bad' blood clots
    (those clots that occlude blood vessels), while sparing hemostatic fibrin
    in the so-called 'good' blood clots (those clots that seal wounds, e.g.,
    after surgery or other tissue injury). Thus, these pro-
UK mutants are excellent and safe thrombolytic agents.
    These advantages allow them to be used in a variety of new methods,
    devices, and compns. useful for thrombolysis and treating various
    cardiovascular disorders in clin. situations where administration of other
    known thrombolytic agents has been too risky or even contraindicated. New
    methods of making the pro-UK mutants are
    also disclosed.
IT  82657-92-9P, Prourokinase
    RL: BPN (Biosynthetic preparation); PAC (Pharmacological activity); THU
    (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES
    (Uses)
      (activated two-chain mutant; mutant
prourokinase compns. for lysis of occlusive blood clots while
sparing wound sealing clots)
IT  56-87-1, Lysine, biological studies
    RL: BSU (Biological study, unclassified); BIOL (Biological study)
      (histidine replacement of; mutant
prourokinase compns. for lysis of occlusive blood clots while
sparing wound-sealing clots)
IT  71-00-1, Histidine, biological studies
    RL: BSU (Biological study, unclassified); BIOL (Biological study)
      (lysine replacement by; mutant prourokinase
compns. for lysis of occlusive blood clots while sparing wound-sealing
clots)
IT  8063-07-8, Kanamycin
    RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
    (Uses)
      (mutant prourokinase compns. for lysis of occlusive
blood clots while sparing wound-sealing clots)

L98 ANSWER 3 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
AN  2004:261901 HCAPLUS
DN  140:387671
TI  Expression and characterization of recombinant human
    amino-terminal fragment of prourokinase
AU  Wang, Jing; Chen, Xin-Yuan; Sun, Zi-Yong; Yao, Hong-Wei; Chen, Jun-Yong;
    Liu, Jian-Ning
CS  Institute of Molecular Medicine, Nanjing University, Nanjing, 210093,

```

- Peop. Rep. China
- SO Nanjing Daxue Xuebao, Ziran Kexue (2004), 40(1), 66-74  
CODEN: NCHPAZ; ISSN: 0469-5097
- PB Nanjing Daxue Xuebao Bianjibu
- DT Journal
- LA Chinese
- AB ATF is the amino terminal Ser1-Lys 135 fragment of **pro-urokinase (proUK)** containing an epidermal growth factor-like (EGF) domain and a kringle domain. It is critically involved in some important functions of **proUK**. The EGF domain participates in receptor binding and promoting cell adhesion and migration. The kringle domain is associated with the chemotactic action, anti-angiogenic and anti-tumor activities. ATF can bind to the **urokinase**-type plasminogen activator receptor (uPAR) with high affinity ( $K_d = 4 \times 10^{-10}$  mol/L) but it is enzymically inactive, and specifically prevent binding of pro-uPA synthesized by tumor cells to the receptors, which inhibits the proliferation and invasion of tumor cells. Fabbrini MS et al. has constructed a **chimera** consisting of the human ATF fused to a cytotoxin saporin isoform (SAP-3) to target and destroy tumor cells. Therefore, ATF is a potential reagent against cancer. Recent studies suggest that ATF also inhibits the replication, assembly and budding of HIV-1, providing a novel therapeutic strategy for AIDS. In this report, the cDNA fragment encoding ATF was cloned from the endothelial cells of human umbilical vein (HUVEC) by reverse transcriptase polymerase chain reaction (RT-PCR). The ATF gene was inserted between the NdeI-XhoI sites of pET-29a (+) vector to construct **recombinant** expression plasmid pET-29a(+)/ATF. The host cell strain **E. coli BL21 (DE3)** transformed with PET-29a(+)/ATF was induced with IPTG to overexpress **recombinant** human ATF (rhATF), as insol. inclusion body. The amount of rhATF expressed accounts for 20% of total bacterial protein. After purification of inclusion body, renaturation, CM cation-exchange chromatog. and Superdex G-75 gel filtration, 15 mg rhATF was obtained from one liter of culture medium with homogeneity greater than 95%. The results of zymograph assay demonstrated that the purified rhATF could block the binding of **proUK** to the uPAR on U973 cell surface. Kinetic anal. showed that both Glu-plasminogen activation by **urokinase** and **proUK** activation by **Lys**-plasmin were dose-dependently inhibited by rhATF.
- IT **82657-92-9P, Prourokinase**  
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)  
(expression and characterization of **recombinant** human amino-terminal fragment of **prourokinase**)
- L98 ANSWER 4 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
- AN 2004:261890 HCAPLUS
- DN 141:64546
- TI Novel kringle **mutant** of **prourokinase** suppressing tumor growth
- AU Cao, Zhong-Wei; Ding, Bi-Sen; Chen, Xin-Yuan; Zhou, Ying-Jiang; Wang, Shi-Quan; Zhang, Jing; Zhu, Zhen-Hua; Chen, Yu-Hong; Liu, Jian-Ning
- CS Institute of Molecular Medicine, Nanjing University, Nanjing, 210093, Peop. Rep. China
- SO Nanjing Daxue Xuebao, Ziran Kexue (2004), 40(1), 28-33  
CODEN: NCHPAZ; ISSN: 0469-5097
- PB Nanjing Daxue Xuebao Bianjibu
- DT Journal
- LA Chinese
- AB Kringles of plasminogen and other proteins, obtained by proteolytic fragments, have been reported to display the anti-tumor activity, which represent potent anti-cancer candidates. However, there remains

controversy on whether it is the sequence or the tertiary structure that renders Kringle the anti-tumor activity. In order to address such an issue, we cloned the genes of Kringle of **prourokinase** and obtained its **mutant** by inserting a previously demonstrated fragment of 16 amino acids from Kringle 5 of plasminogen that manifested anti-tumor activity. The constructed **recombinant** vectors **pET29a** were expressed in **E. coli BL21 (DE3)**, induced by IPTG. **Prourokinase** Kringle and the **mutant** were first purified by Ni-NTA affinity chromatog. and then subjected to renaturation. Finally, the folding solns. were applied to CM ion-exchange chromatog. for further purification and concentration As a result,

appropriately folded proteins with high purity were obtained, which were confirmed by SDS-PAGE anal. To compare the in vivo anti-tumor activities of **prourokinase** Kringle and its **mutant**, male 6-wk C57/BL6 mice were used for tumor study. Lewis lung carcinoma cells were s.c. injected and the anti-tumor efficacy was evaluated on the basis of tumor volume Here, **prourokinase** Kringle almost displayed no anti-tumor activity while its **mutant** comparatively stifled the growth of s.c. tumor, illustrating that equipping proteins with certain anti-tumor fragment will inhibit tumor growth and it is the amino acid sequence rather than the tertiary structure of protein that enables several Kringle structures to prevent tumor from growing.

IT 82657-92-9, **Prourokinase**

RL: PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)  
(novel kringle **mutant** of **prourokinase** suppressing tumor growth)

L98 ANSWER 5 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:907166 HCAPLUS

DN 138:322

TI Plasma glucosylceramide deficiency as risk factor for thrombosis and modulator of anticoagulant protein C

IN Griffin, John H.; Deguchi, Hiroshi; Fernandez, Jose

PA USA

SO U.S. Pat. Appl. Publ., 32 pp.

CODEN: USXXCO

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2002177563	A1	20021128	US 2002-86943	20020228 <--
	US 6756208	B2	20040629		
	WO 2002102325	A2	20021227	WO 2002-US6340	20020228 <--
	WO 2002102325	A3	20030912		
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW			
	RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
	EP 1370570	A2	20031217	EP 2002-760992	20020228 <--
	R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR			
	US 2004132688	A1	20040708	US 2003-739962	20031217 <--
PRAI	US 2001-272103P	P	20010228	<--	
	US 2001-278045P	P	20010322	<--	

US 2002-86943 A3 20020228 &lt;--

WO 2002-US6340 W 20020228 &lt;--

AB The present invention has determined that exogenously added glucosylceramide (GlcCer) and other neutral glycolipids such as the homologous Glc-containing globotriaosylceramide (Gb3Cer), dose-dependently prolonged clotting times of normal plasma in the presence but not absence of APC:protein S, indicating GlcCer or Gb3Cer can enhance protein C pathway anticoagulant activity. In studies using purified proteins, inactivation of factor Va by APC:protein S was enhanced by GlcCer alone and by GlcCer, globotriaosylceramide, lactosylceramide, and galactosylceramide in multicomponent vesicles containing phosphatidylserine and phosphatidylcholine. Thus, the present invention provides neutral glycolipids such as GlcCer and Gb3Cer, as anticoagulant cofactors that contribute to the antithrombotic activity of the protein C pathway. The present invention has also determined that a deficiency of plasma GlcCer is a risk factor for thrombosis. Methods are provided to determine individuals at risk for thrombosis, methods of treatment as well as methods of screening for antithrombotic factors from neutral glycolipids.

IT 71965-57-6, Globotriaosylceramide

RL: ANT (Analyte); DGN (Diagnostic use); PAC (Pharmacological activity); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)

(plasma glucosylceramide or other neutral glycolipid deficiency as risk factor for thrombosis and modulator of anticoagulant protein C when given in vesicle form in relation to combination with other agents)

IT 82657-92-9, Prourokinase 82657-92-9D,

Prourokinase, analogs

RL: PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(plasma glucosylceramide or other neutral glycolipid deficiency as risk factor for thrombosis and modulator of anticoagulant protein C when given in vesicle form in relation to combination with other agents)

## RETABLE

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	Referenced File
=====	=====	=====	=====	=====	=====
Anon	1998			WO 9856365 A1	HCAPLUS
Anon	2000			WO 0053264 A1	HCAPLUS
Chatterjee, S	1998	18	1523	Arterioscler Thromb	HCAPLUS
Clarke, J	1981	59	412	Can J Biochem.	HCAPLUS
Dawson, G	1976	17	125	J Lipid Res.	HCAPLUS
Debuchi, H	2001	97	1907	Blood	
Deguchi, H	2002	277	8861	J Biol Chem	HCAPLUS
Deguchi, H	2000	97	1743	Proc. Natl Acad Sci	MEDLINE
Fernandez, J	2000	26	115	Blood Cells Mol Dis.	HCAPLUS
Griffin, J	1999	103	219	J. Clin Invest.	HCAPLUS
Hakomori, S	1995	118	1091	J Biochem.	HCAPLUS
Heran, C	2000	389	201	Eu. J. Pharm.	HCAPLUS
Phillips, D	1993	61	344	J. Neurochem.	HCAPLUS
Smirnov, M	1994	269	816	J Biol Chem.	HCAPLUS
Svensson, P	1994	330	517	N Engl J. Med.	MEDLINE

L98 ANSWER 6 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:326876 HCAPLUS

DN 137:273001

TI Prourokinase mutant that induces highly effective clot lysis without interfering with hemostasis

AU Liu, Jian-Ning; Liu, Jian-Xia; Liu, Bei-fang; Sun, Ziyong; Zuo, Jian-Ling; Zhang, Pei-xiang; Zhang, Jing; Chen, Yu-hong; Gurewich, Victor

CS Vascular Research Laboratory, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

SO Circulation Research (2002), 90(7), 757-763

CODEN: CIRUAL; ISSN: 0009-7330

PB Lippincott Williams & Wilkins

DT Journal

LA English

AB **Prourokinase (proUK)** is a zymogenic plasminogen activator that at pharmacol. doses is prone to nonspecific activation to **urokinase**. This has handicapped therapeutic exploitation of its fibrin-specific physiol. properties. To attenuate this susceptibility without compromising specific activation of **proUK** on a fibrin clot, a **Lys 300 → His** **mutation (M5)** was developed. M5 had a lower intrinsic activity and, therefore, remained stable in plasma at a 4-fold higher concentration than did **proUK**. M5 had a higher 2-chain activity and induced more rapid plasminogen activation and fibrin-specific clot lysis in vitro. Sixteen dogs embolized with radiolabeled clots were infused with saline, **proUK**, tissue plasminogen activator, or M5. The lower intrinsic activity allowed a higher infusion rate with M5, which induced the most rapid and efficient clot lysis (50% clot lysis by ≈600 µg/kg M5 vs. ≈1200 µg/kg **proUK**). In association with this, M5 caused neither a significant increase in the primary bleeding time nor secondary bleeding (total blood loss). By contrast, these measurements increased 4-fold and 5-fold, resp., with **proUK** and >5-fold and 8-fold, resp., with tissue plasminogen activator. Clot lysis by M5 and hemostasis were further evaluated in 6 rhesus monkeys. M5 again induced rapid clot lysis without a significant increase in the primary bleeding time, and secondary bleeding did not occur. In conclusion, a site-directed **mutation** designed to improve the stability of **proUK** in blood at therapeutic concns. induced superior clot lysis in vitro and in vivo without causing significant interference with hemostasis.

IT **82657-92-9, Prourokinase**

RL: PAC (Pharmacological activity); BIOL (Biological study)  
(comparison with; **prourokinase mutant** that induces highly effective clot lysis without interfering with hemostasis)

IT **82657-92-9DP, Prourokinase, histidine 300**

**mutant, M5**

RL: BSU (Biological study, unclassified); PAC (Pharmacological activity); PRP (Properties); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)  
(**prourokinase mutant** that induces highly effective clot lysis without interfering with hemostasis)

# RETABLE

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	Referenced File
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Carmeliet, P	1994	368	419	Nature	HCAPLUS
Collen, D	1985	72	384	Circulation	MEDLINE
Declerck, P	1991	64	394	Thromb Haemost	
Gurewich, V	1984	73	1731	J Clin Invest	HCAPLUS
Gurewich, V	1988	82	1956	J Clin Invest	HCAPLUS
Harpel, P	1985	260	4432	J Biol Chem	HCAPLUS
Hekman, C	1988	27	2911	Biochemistry	HCAPLUS
Lang, I	1993	87	1990	Circulation	HCAPLUS
Liu, J	1992	31	6311	Biochemistry	HCAPLUS
Liu, J	1996	35	14070	Biochemistry	HCAPLUS
Liu, J	1993	81	980	Blood	HCAPLUS
Liu, J	1992	267	15289	J Biol Chem	HCAPLUS
Liu, J	1995	270	8408	J Biol Chem	HCAPLUS
Liu, J	1991	88	2012	J Clin Invest	HCAPLUS
Meyer, J	1989	1	863	Lancet	
Michels, H	1996	7	766	Blood Coagul Fibrinol	HCAPLUS
Michels, R	1995	2	117	J Thromb Thrombolysis	HCAPLUS
Montoney, M	1995	91	1540	Circulation	HCAPLUS
Northeast, A	1995	22	573	J Vasc Surg	MEDLINE

Orini, G	1991	195	691	Eur J Biochem	
Pannell, R	1986	67	1215	Blood	HCAPLUS
Pannell, R	1987	69	22	Blood	HCAPLUS
Pannell, R	1988	81	853	J Clin Invest	HCAPLUS
Sun, Z	1998	37	2935	Biochemistry	HCAPLUS
Sun, Z	1997	272	23818	J Biol Chem	HCAPLUS
Swaim, W	1967	13	1026	Clin Chem	HCAPLUS
Topol, E	1993	329	673	N Engl J Med	
Torr, S	1992	19	1085	J Am Coll Cardiol	HCAPLUS
Van de Werf, F	1984	69	605	Circulation	HCAPLUS
Verde, P	1984	81	4727	Proc Nat Acad Sci	HCAPLUS
Winkler, M	1986	25	4041	Biochemistry	HCAPLUS
Yamamoto, K	1996	97	2440	J Clin Invest	HCAPLUS

L98 ANSWER 7 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:52047 HCAPLUS

DN 136:339530

TI Characterization and large scale preparation of human **recombinant prourokinase**

AU Chen, Yuhong; Sun, Ziyong; Zhu, Zhenhua; Zhang, Jing; Fu, Yigong; Zhu, Dexu; Liu, Jianning

CS Institute of Molecular Medicine, Nanjing University, Nanjing, 210093, Peop. Rep. China

SO Nanjing Daxue Xuebao, Ziran Kexue (2001), 37(4), 407-415

CODEN: NCHPAZ; ISSN: 0469-5097

PB Nanjing Daxue Xuebao Bianjibu

DT Journal

LA Chinese

AB The engineered *E. coli* containing **pET29a-prouk** was cultured in a 10 L seeding tank, and then grew in a 100 L fermentor under IPTG induction. The expressed protein accounted for 20% of the bacteria total proteins. After renaturation, the folding solution was applied to a column of CM-cellulose, and the fraction containing **recombinant prourokinase** activity further was purified by Superdex 75 gel filtration and depynogenated by Affiprep polymyxin affinity chromatog. A pilot purification yielded 6 g of purified human **recombinant prourokinase** from 100 L medium. The purity of the resulting protein was higher than 95 %, and its specific activity was over 120 000 iu/mg. The content of the two chain **urokinase** was less than 0.5 %, and the trace content of pyrogen, the residual protein and DNA from the host cell all met the requirements for clin. use. The mol. weight of the **recombinant prourokinase** and the amino acid composition were consistent with the theor. data. The isoelectrophoretic point and peptide mapping of the **pro-urokinase** were also determined

IT 82657-92-9P, **Prourokinase**

RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation) (characterization and large scale preparation of human **recombinant prourokinase**)

L98 ANSWER 8 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:52046 HCAPLUS

DN 137:89065

TI Human **prourokinase** cDNA gene cloning and engineered strain construction and characterization

AU Chen, Yuhong; Zhang, Jing; Zhu, Zhenhua; Fu, Yigong; Liu, Jianning

CS Institute of Molecular Medicine, Nanjing University, Nanjing, 210093, Peop. Rep. China

SO Nanjing Daxue Xuebao, Ziran Kexue (2001), 37(4), 401-406

CODEN: NCHPAZ; ISSN: 0469-5097

PB Nanjing Daxue Xuebao Bianjibu

DT Journal

- LA Chinese  
AB The human **recombinant prourokinase** gene was cloned from the epithelial cells of human umbilical vein by RT-PCR. The **pET29a/prouk** plasmid was constructed and expressed in **Escherichia coli BL21(DE3)** strain by IPTG induction. The expression product with a 46 kDa was identified by SDS-PAGE, and the protein accounted for 20% of the bacterial total protein as inclusion body. The engineered strain showed the stability of its plasmid maintenance, resuscitation and expression efficiency in storage and regeneration.
- IT **82657-92-9, Prourokinase**  
RL: BSU (Biological study, unclassified); BIOL (Biological study) (human **prourokinase** cDNA gene cloning and engineered strain construction and characterization)
- L98 ANSWER 9 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 2002:20281 HCAPLUS  
DN 136:397679  
TI Characterization of bifunctional **chimeric** molecule of PRGDWR containing **pro-urokinase**  
AU Dang, Xin; Yang, Jingxin; Ru, Qiang; Ru, Binggen  
CS National Laboratory of Protein Engineering, College of Life Sciences, Peking University, Beijing, 100871, Peop. Rep. China  
SO Shengwu Huaxue Yu Shengwu Wuli Jinzhan (2001), 28(2), 203-209  
CODEN: SHYCD4; ISSN: 1000-3282  
PB Shengwu Huaxue Yu Shengwu Wuli Jinzhan Bianjibu  
DT Journal  
LA Chinese  
AB The bifunctional **chimeric** mol. of **single-chain urokinase**-type plasminogen activator (**scu-PA**) which inhibits platelet aggregation was studied. The PRGDWR peptide was inserted into the site between Gly118 and Leu119 (called insertion **mutant B**, InB). The **recombinant** gene of InB was expressed by *Pichia pastoris*. The secreted protein was purified by metal chelate affinity and strong cation exchange chromatog. The amidolytic ability of **mutant** InB is 5900 IU/mg, the kinetic consts. is:  $K_m, plgInB = 56.8 \mu\text{mol L}^{-1}$ ,  $k_{cat}, plgInB = 0.33 \text{ s}^{-1}$ . The kinetic consts. of plasminogen activation reaction is:  $KInBm, plg = 0.397 \mu\text{mol L}^{-1}$ ,  $kInBcat, plg = 0.0164 \text{ s}^{-1}$ . Fibrin inhibit the catalytic ability of InB during plasminogen activation, the influence factor is 0.463 (means InB remain 46.3% of the catalytic ability when fibrin was involved in the reaction system). The **mutant** not only has almost the same catalytic ability as wild type **scu-PA**, but also has strong ability of anti-platelet aggregation (compared with **scu-PA**),  $IC_{50}$  of InB is  $12.7 \mu\text{mol L}^{-1}$ .
- IT **82657-92-9DP, Single-chain urokinase**  
-type plasminogen activator, PRGDWR-containing analogs  
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); CAT (Catalyst use); PRP (Properties); BIOL (Biological study); PREP (Preparation); USES (Uses)  
(characterization of bifunctional **chimeric** mol. of PRGDWR containing **pro-urokinase**)
- L98 ANSWER 10 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 2001:461655 HCAPLUS  
DN 136:194764  
TI Application of gel chromatography renaturing way on low molecular **single-chain urokinase mutant** (DscuPA-32K)  
AU Jiao, Jianwei; Yu, Meimin; Ru, Binggen  
CS National Laboratory of Protein Engineering, College of Life Sciences, Peking University, Beijing, 100871, Peop. Rep. China  
SO Shengwu Gongcheng Xuebao (2001), 17(3), 300-303

CODEN: SGXUED; ISSN: 1000-3061

PB Kexue Chubanshe

DT Journal

LA Chinese

AB The application of gel chromatog. renaturing method on low mol.

**single-chain urokinase mutant**

(DscuPA-32K) was studied. A recombinant mutant gene with thrombolytic and antithrombolytic bifunction was expressed in *E. coli*. Owing to two reasons of high mol. weight and over expression, dscuPA existed in inclusion body form. The protein of inclusion body was inactive protein. To obtain active protein, inclusion bodies should be denatured and then renatured. A novel, named gel-chromatog. column renaturation method was performed. Compared with traditional renaturation method, this refolding approach had obvious advantages including low cost and high recovery.

IT 82657-92-9, **Single-chain urokinase**

RL: PEP (Physical, engineering or chemical process); PROC (Process)

(application of gel chromatog. renaturing method on low mol.

**single-chain urokinase mutant**

(DscuPA-32K))

L98 ANSWER 11 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2001:350846 HCAPLUS

DN 135:16011

TI Catalysis-related function of kringle domain of **single chain urokinase**-type plasminogen activator

AU Dang, Xin; Ji, Jian-guo; Ru, Qiang; Yang, Jing-xin; Yu, Mei-min; Ru, Bing-gen

CS National Lab. of Protein Engineering, Peking University, Beijing, 100871, Peop. Rep. China

SO Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (2001), 17(2), 219-225

CODEN: ZSHXF2; ISSN: 1007-7626

PB Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui

DT Journal

LA Chinese

AB Kringle domain is a conservative domain that exists in many thrombus forming and thrombolysis related factors. The primary structure of Kringle domain is high conserved (compared with other domains in those factors). To study the function of Kringle domain of **single chain urokinase**-type plasminogen activator (**scu-PA**), an Kringle domain inserted **mutant** of **scu-PA** was constructed by inserting PRGDWR peptide at the site between Gly118 and Leu119 (Insert **mutant B**, InB) and the kinetic consts. of thrombolytic related reactions were detected. Km value of hydrolytic reaction of S-2444 catalyzed by the two mol. was not significantly different (60.4 and 56.8  $\mu\text{mol}\cdot\text{L}^{-1}$  resp.), but the Km value of InB (0.33 s<sup>-1</sup>) was decreased a lot compared with the kcat of **scu-PA** (7.31 s<sup>-1</sup>). The Km value of InB (0.397  $\mu\text{mol}\cdot\text{L}^{-1}$ ) of the activation reaction of plasminogen was 40% lower than the Km value of **scu-PA** (0.648  $\mu\text{mol}\cdot\text{L}^{-1}$ ), but the kcat value of InB (0.0165 s<sup>-1</sup>) was over 70% lower than that of **scu-PA** (0.0626 s<sup>-1</sup>). These results suggest that Kringle domain of **scu-PA** is related with the catalytic activity of **scu-PA**, but has no influence on the affinity between **scu-PA** and its substrate.

IT 82657-92-9, **Single chain urokinase**

-type plasminogen activator

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)

(Kringle domain of **single chain urokinase**

-type plasminogen activator (**scu-PA**) is related

with the catalytic activity of **scu-PA**, but has no

influence on affinity between **scu-PA** and its substrate)

- L98 ANSWER 12 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 2001:191009 HCAPLUS  
DN 135:339965  
TI Thioredoxin reductase-deficient **E. coli mutant** enhances expression into solution of **recombinant** proteins containing Cys residues  
AU Tong, Qin; Yang, Yungui; Zhang, Huitang; Chen, Yan; Yang, Shengli; Gong, Yi  
CS Shanghai Research Center of Biotechnology, Chinese Academy of Sciences, Shanghai, 200233, Peop. Rep. China  
SO Shengwu Huaxue Yu Shengwu Wuli Xuebao (2001), 33(1), 30-34  
CODEN: SHWPAU; ISSN: 0582-9879  
PB Shanghai Kexue Jishu Chubanshe  
DT Journal  
LA Chinese  
AB A 3D artificial protein, a salmon calcitonin hexapolymer, a salmon calcitonin octopolymer, and a human **prourokinase**, was expressed in cytoplasm of **E.coli GJ980 (trxB-) mutant**. The **recombinant** proteins contained cysteine residues of different length of 12-22 residues. The **mutation** was mapped to the gene for thioredoxin reductase and may eliminate the activity of enzyme, and the effect was related to the sulfhydryl reducing potential of cytoplasm. **Recombinant** salmon calcitonin hexapolymer, salmon calcitonin octopolymer, and human **prourokinase** had more soluble form in cytoplasm of GJ980 **mutants** than in wild-type strain, but 3D-protein with no cysteine residue remained in insol. form. The results showed that the formation of disulfide bonds in cell cytoplasm GJ980 (trxB-) strain may play an important role in form correct folding and soluble expression of the **recombinant** proteins.
- IT **82657-92-9P, ProUrokinase**  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(human; thioredoxin reductase-deficient **E. coli mutant** enhances expression into solution of **recombinant** proteins containing Cys residues)
- L98 ANSWER 13 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 2001:124859 HCAPLUS  
DN 135:252488  
TI Studies on expression of human **pro-urokinase** in **Escherichia coli**  
AU Wang, Tao; Zhou, Xianwan; Hu, Meihao  
CS College Life Sciences, Peking University, Beijing, 100871, Peop. Rep. China  
SO Beijing Daxue Xuebao, Ziran Kexueban (2000), 36(6), 802-807  
CODEN: PCTHAP; ISSN: 0479-8023  
PB Beijing Daxue Chubanshe  
DT Journal  
LA Chinese  
AB Studies were made on the enhancement of expression of human **pro-Urokinase(Pro-UK)** cDNA in **Escherichia coli**. By means of PCR, the signal peptide DNA sequence was deleted. For study of the limited factors in expression of **pro-Urokinase**, the **pro-UK** gene was divided into three fragments, and they were expressed in **E. coli BL21(DE3)**. The expression of middle fragment is very low, because there are several rare codon AGG (Arg) in it. Using a new bacteria **E. coli BL21-Codon Plus-RIL** to introduce DNA Y gene coding tRNAagg/aga (Arg) to recognize the rare

codon AGG, the expression level of the middle fragment was enhanced to 10%-20% of total cell protein and the expression of the intact **pro-UK** was enhanced to 5% by more than 10 folds.

IT **82657-92-9P, Pro-Urokinase**

RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)

(expression of human **pro-urokinase** in **Escherichia coli**)

L98 ANSWER 14 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2000:664901 HCAPLUS

DN 134:1977

TI A region in domain II of the **urokinase** receptor required for **urokinase** binding

AU Bdeir, Khalil; Kuo, Alice; Mazar, Andrew; Sachais, Bruce S.; Xiao, Weizhong; Gawlak, Susan; Harris, Scott; Higazi, Abd Al-Roof; Cines, Douglas B.

CS Departments of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, 19104, USA

SO Journal of Biological Chemistry (2000), 275(37), 28532-28538  
CODEN: JBCHA3; ISSN: 0021-9258

PB American Society for Biochemistry and Molecular Biology

DT Journal

LA English

AB The **urokinase** receptor is composed of three homologous domains based on disulfide spacing. The contribution of each domain to the binding and activation of **single chain urokinase** (scuPA) remains poorly understood. In the present paper we examined the role of domain II (DII) in these processes. Repositioning DII to the amino or carboxyl terminus of the mol. abolished binding of scuPA as did deleting the domain entirely. By using alanine-scanning **mutagenesis**, we identified a 9-amino acid continuous sequence in DII (Arg137-Arg145) required for both activities. Competition-inhibition and surface plasmon resonance studies demonstrated that **mutation** of Lys139 and His143 to alanine in soluble receptor (suPAR) reduced the affinity for scuPA .apprx.5-fold due to an increase in the "off rate". **Mutation** of Arg137, Arg142, and Arg145, each to alanine, leads to an .apprx.100-fold decrease in affinity attributable to a 10-fold decrease in the apparent "on rate" and a 6-fold increase in off rate. These differences were confirmed on cells expressing variant **urokinase** receptor. SuPARK139A/H143A displayed a 50% reduction in scuPA-mediated plasminogen activation activity, whereas the 3-arginine variant was unable to stimulate scuPA activity at all. **Mutation** of the three arginines did not affect binding of a decamer peptide antagonist of scuPA known to interact with DI and DIII. However, this **mutation** abolished both the binding of soluble DI to DII-III in the presence of scuPA and the synergistic activation of scuPA mediated by DI and wild type DII-DIII. These data show that DII is required for high affinity binding of scuPA and its activation. DII does not serve merely as a spacer function but appears to be required for interdomain cooperativity.

IT **71-00-1, L-Histidine**, biological studies

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)

(His143; 9-amino acid sequence in domain II of **urokinase** receptor (Arg137-Arg145) required for **urokinase** binding)

IT **56-87-1, L-Lysine**, biological studies

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)

(Lys139; 9-amino acid sequence in domain II of **urokinase** receptor (Arg137-Arg145) required for **urokinase** binding)

IT **82657-92-9, Single chain urokinase**

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)

(region in domain II of **urokinase** receptor required for **urokinase** binding)

- L98 ANSWER 15 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 2000:363770 HCAPLUS  
DN 133:291675  
TI Construction of **pro-urokinase mutant**  
Glu151-Glu154-mscu-PA and characterization of its kinetic properties  
AU Liu, Wei; Zhu, Hui; Shi, Wei; Ma, Zhong  
CS Department of Biochemistry and State Laboratory of Pharmaceutical  
Biotechnology, Nanjiang University, Nanjiang, 210093, Peop. Rep. China  
SO Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (2000), 16(2),  
188-193  
CODEN: ZSHXF2; ISSN: 1007-7626  
PB Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui  
DT Journal  
LA Chinese  
AB Rscu-PA and its **mutant** constructed in vitro by site-directed  
**mutagenesis** of Lys151, Arg154 in rscu-PA to Glu151,  
Glu154(mscu-PA) were both expressed in **Escherichia coli**  
. After in vitro denaturation and renaturation, the rscu-PA and mscu-PA  
were purified to homogeneity by Zn<sup>2+</sup> selective precipitation, anti-u-PA  
IgG-Sepharose CL 4B affinity chromatog. The activation by plasmin of  
mscu-PA was 40% lower than that of rscu-PA. Mscu-PA and rscu-PA were  
found essentially identical in Glu-plasminogen activation. After  
activation by plasmin, the kinetic consts. for the resultant mtcu-PA  
against synthetic substrate S2444 hydrolysis were found about 90% of  
rtcu-PA. Although the Km of two-chain mtcu-PA against Glu-plasminogen was  
similar to that of rtcu-PA, mtcu-PA had a lower enzymic activity(about 80%  
that of rtcu-PA) due to a reduction of Kcat. In caseinolytic system, with the  
fibrin and plasminogen, mscu-PA could speed up the decomposition of casein more  
than rscu-PA, which suggested that mtcu-PA had some fibrin-specificity.
- L98 ANSWER 16 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 2000:344507 HCAPLUS  
DN 133:291639  
TI High level expression of a **mutant** (K151E, R154G) of  
**single chain urokinase**-type plasminogen  
activator in silkworm  
AU Peng, Rui; Yu, Zhe-yong; Zang, Yu-hui; Qin, Jun-chuan  
CS Department of Biochemistry and State Key Laboratory of Pharmaceutical  
Biotechnology, Nanjing University, Nanjing, 210093, Peop. Rep. China  
SO Nanjing Daxue Xuebao, Ziran Kexue (2000), 36(2), 235-238  
CODEN: NCHPAZ; ISSN: 0469-5097  
PB Nanjing Daxue  
DT Journal  
LA English  
AB The cDNA of a **mutant of single chain**  
**urokinase**-type plasminogen activator (rscu-PA) gene in silkworm,  
mscu-PA (K151E, R154G), was reconstructed to include the natural  
**urokinase** (u-PA or UK) signal peptide sequences, which was  
obtained by the PCR method. In order to introduce the whole mscu-PA gene  
(including the signal peptide sequence) into BmNPV genome and put it under  
the control of the polyhedrin (ph) promoter, a BmNPV-derived transfer  
vector pBE284 was used as well as pVL1392, a transfer vector derived from  
AcNPV. Based on the sequence homol. of the ph promoter and its flanking  
region in BmNPV and AcNPV genome, the 5' non-encoding sequence (including  
promoter) of the ph gene from pVL1392 replaced that in wild type BmNPV DNA  
via in vivo homologous **recombination**. The two polyhedrin-  
**recombinant** viruses were isolated and identified by dot  
hybridization using a labeled **scu-PA** cDNA probe, and  
were designated BmNPV-mscu-PA-B (by pBE284) and BmNPV-mscu-PA-V  
(by pVL1392), resp. The protein mscu-PA was secreted into both the

culture medium and hemolymph of the larvae, and exhibited high biol. activity. The highest yield of the **recombinant** protein was about 150 µg/mL. Furthermore, compared with those infected with BmNPV-mscu-PA-B, the yield of msu-PA was increased threefold in cultured BmN cells and 2.3 times in silkworm larvae infected with BmNPV-mscu-PA-V. The highest expression may be due to the structure of pVL1392. In pVL1392, the entire 5'-untranslated region of (UTR) ph gene is present as well as the first 35 nucleotides of the ph coding sequences, except that ATG has been modified to ATT to prevent the synthesis of a fusion protein. This may play an important role in the translation of the **recombinant** protein in insect cells. It may increase the RNA stability and translational efficiency by resembling the 5' portion of the natural ph mRNA or by forming a longer 5' UTR. The transfer vector pVL1392 and the silkworm expression system could be used as one of the most economical ways of overproducing msu-PA protein.

IT **82657-92-9P, Single chain urokinase**

-type plasminogen activator

RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)

(high level expression of a **mutant** (K151E, R154G) of **single chain urokinase**-type plasminogen activator in silkworm)

RETABLE

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	Referenced File
Jarvis, D	1990	42	181	J Cell Biochem	HCAPLUS
Meads, S	1989	I	167	Invertebrate Cell Sy	
O'Reilly, D	1992			A Laboratory Manual	
Peng, G	1997	42	972	Chinese Science Bull	

L98 ANSWER 17 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2000:344480 HCAPLUS

DN 133:131555

TI Construction and characterization of two **mutants** of **pro**-**urokinase** (Ala175 →Ser, Tyr187→**His** and Ala175→Ser, Tyr187→**His**)

AU Shi, Wei; Zhu, Hui; Xue, Yu-ming; Tang, Tang; Ma, Zhong

CS Department of Biochemistry, National Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, Peop. Rep. China

SO Nanjing Daxue Xuebao, Ziran Kexue (2000), 36(2), 208-212

CODEN: NCHPAZ; ISSN: 0469-5097

PB Nanjing Daxue

DT Journal

LA Chinese

AB Human **single chain urokinase**-type

plasminogen activator (**scu-PA**, also named **pro**

**-UK**) is an important thrombolytic agent in therapy of

thrombosis. The activation of plasminogen on the surface of fibrin

induced by **pro-UK**, is much specific and effective,

resulting very small tendency of bleeding from system lytic state.

Therefore, great importance has been attached to **scu-PA**

in clinics. Though **pro-UK** has some selectivity for

fibrin, its higher selectivity for fibrin in human bodies was counteracted

by its higher intrinsic activity when it was used in large doses. The

risk of bleeding still remains. It was discovered that the stretch of

297.apprx.313 amino acids in **scu-PA** formed a loop.

**Lys300**, the only pos. charge amino acid in the loop might interact

with Asp355 which was sited near the active center by charge attraction.

Thus Ser365 was pulled, Ser365 Asp225, His204 might form the active center

on the 3-D structure resulted in the high intrinsic activity of

**pro-UK**. Besides, according to the data of crystal

structure of chymotrypsin, Asp194, His40and Ser32 formed a zymogen triad,

which keeps the inactive conformation of chymotrypsin. As a member of the zymogens of serine protease family, **pro-UK** lacks the zymogen triad, His40 and Ser32 were replaced resp. by Tyr187 and Ala175. This was also the structure basis of **pro-UK**'s high enzymic activity. To reduce the intrinsic activity of **pro-UK**, 2 mutant genes of **pro-urokinase**, muk1 (Ala175→Ser, Tyr187.fwdarw .His, Lys300.fwdarw.His) and muk2 (Ala175→Ser, Tyr187→His) were constructed by site directed mutagenesis, and were expressed in *E. Coli* BL21. The expressed inclusion body was treated by denaturation and renaturation, and purified by SP-sepharose ion-exchange chromatog. and Benzamidine Sepharose affinity adsorption. Using the synthetic substrate S2444, the intrinsic activity and the enzymic activity of two-chain form of muk1 and muk2 were measured. The intrinsic activities of muk1 and muk2 were 8-fold and 2.5-fold lower than that of **pro-urokinase**, resp. The enzymic activity of two-chain muk1 was 1.5-fold higher than that of **urokinase** and the activity of muk2 was the same as wild type **urokinase**. The mechanism and the structure basis of a much higher intrinsic catalytic activity than other zymogens of the serine protease family was discussed.

IT **82657-92-9P, Single-chain urokinase**

-type plasminogen activator

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PEP (Physical, engineering or chemical process); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation); PROC (Process)

(construction and characterization of two mutants (muk1 and muk2) of **pro-urokinase** (Ala175 →Ser, Tyr18.fwdarw.His and Ala175→Ser, Tyr187→His))

L98 ANSWER 18 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2000:141655 HCAPLUS

DN 133:39761

TI Construction and characterization of a mutant of single-chain urokinase-type plasminogen activator (Ser175-His187-mscu-PA)

AU Xue, Yu-Ming; Zhu, Hui; Shi, Wei; Liu, Wei; Liu, Jian-Ning; Ma, Zhong

CS Department of Biochemistry, National Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, Peop. Rep. China

SO Shengwu Huaxue Yu Shengwu Wuli Xuebao (2000), 32(1), 26-30

CODEN: SHWPAU; ISSN: 0582-9879

PB Shanghai Kexue Jishu Chubanshe

DT Journal

LA Chinese

AB **Single-chain urokinase-type plasminogen**

activator (**scu-PA**) is the precursor of double-chain

**urokinase** (**tcu-PA**), which has a much higher intrinsic catalytic

activity than other zymogens of the serine protease family. To restore

the "zymogen triad" of Asp-His-Ser in the serine protease

family, the mutant gene of **scu-PA** (**mscu-PA**,

Ala175 → Ser175, Tyr187 → His187) was constructed by the

method of oligonucleotide-directed, site-specific mutagenesis in

order to reduce its intrinsic catalytic activity. **mscu-PA** was expressed in *E. coli* BL21. After denaturation and

renaturation in vitro, the **mscu-PA** was purified to homogeneity by

SP-Sepharose ion-exchange chromatog., Sephacryl S-200 chromatog. and

Benzamidine-Sepharose affinity adsorption, and the mutant

**mscu-PA** had the same activity to plasmin as **scu-PA**.

The catalytic efficiency (measured by kcat/Km) of the mutant to

synthetic substrate S2444 was 2.5-fold lower than that of **scu-**

**PA**, and the activity against Glu-plasminogen was also reduced.

After activation by plasmin, mtcu-PA and tcu-PA had similar catalytic efficiency against S2444 and Glu-plasminogen. The intrinsic catalytic activity of mscu-PA may be reduced after restoring the "zymogen triad".

L98 ANSWER 19 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
 AN 2000:55660 HCAPLUS  
 DN 132:203955  
 TI The expression of **proUK** in **Escherichia coli**:  
 the vgb promoter replaces IPTG and coexpression of argU compensates for rare codons in a hypoxic induction model  
 AU Jiang, Lan; Yang, Yonghua; Chatterjee, Shampa; Seidel, Bertolt; Wolf, Gerald; Yang, Shengli  
 CS Shanghai Research Center of Biotechnology, Chinese Academy of Sciences, Shanghai, 200233, Peop. Rep. China  
 SO Bioscience, Biotechnology, and Biochemistry (1999), 63(12), 2097-2101  
 CODEN: BBBIEJ; ISSN: 0916-8451  
 PB Japan Society for Bioscience, Biotechnology, and Agrochemistry  
 DT Journal  
 LA English  
 AB The expression of the **proUK** gene was improved by the coexpression of the argU gene cloned in a moderate copy number vector. As the **proUK** gene contains 2% AGG/AGA codons, which is much higher than the normal frequency in **E. coli**, about 0.14-0.21%, the argU gene cloned in a multicopy plasmid was coexpressed with the **proUK** expression vector in these expts. In **E. coli** strain BL21(DE3), IPTG is known to induce the expression of T7 RNA polymerase gene and this enzyme can transcribe the **proUK** gene under the control of the T7 promoter leading to expression of **proUK**. To replace IPTG by a cheaper alternative on a large scale, a plasmid was constructed in which the vgb promoter - which is known to be activated by the onset of hypoxic conditions - controls T7 RNA polymerase gene expression. Low oxygen conditions were then used to activate the vgb promoter causing T7 RNA polymerase gene expression and finally leading to the expression of **proUK** as inactive inclusion bodies. These expts. on a large scale in a bioreactor show that the expression of **proUK** accounts for .apprx.30% of total protein after about 6 h of anaerobic cultivation, so the presented model represents an economical alternative to IPTG induction.  
 IT 82657-92-9P, Pro-urokinase  
 RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)  
 (expression of **proUK** in **Escherichia coli** so that the vgb promoter replaces IPTG and coexpression of argU compensates for rare codons in a hypoxic induction model)

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Bruke, S	1995	77	1025	Thromb Haemost	
Calderone, T	1996	262	407	J Mol Biol	HCAPLUS
Chen, G	1994	8	2641	Genes Dev	HCAPLUS
Credo, R	1995	6	85	J Vasc Interv Radiol	MEDLINE
Di Minno, G	1989	21	153	Pharmacol Res	MEDLINE
Dikshit, K	1988	70	377	Gene	HCAPLUS
Dikshit, K	1989	135	2601	J Gen Microbiol	HCAPLUS
Dikshit, K	1989	135	2601	J Gen Microbiol	HCAPLUS
Dikshit, K	1990	18	4149	Nucleic Acids Res	HCAPLUS
Hua, Z	1996	220	131	Biochem Biophys Res	HCAPLUS
Joshi, M	1994	202	535	Biochem Biophys Res C	HCAPLUS
Li, F	1995	7	113	Chin J Biotechnol	

Saxena, P	1992	174	1956	J Bacteriol	HCAPLUS
Spanjaard, R	1990	18	5031	Nucleic Acids Res	HCAPLUS
Spanjaard, R	1988	85	7967	Proc Natl Acad Sci	HCAPLUS
Spiecker, M	1994	19	326	Herz	MEDLINE
Vieira, J	1991	100	189	Gene	HCAPLUS
Wang, R	1991	100	195	Gene	HCAPLUS

L98 ANSWER 20 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1999:816585 HCAPLUS

DN 132:289346

TI Construction and expression of a **recombinant** antibody-targeted plasminogen activator

AU Yang, Jiashu; Jiang, Pengchen; Ru, Binggen

CS National Laboratory of Protein Engineering and Plant Genetic Engineering, College of Life Sciences, Peking University, Beijing, 100871, Peop. Rep. China

SO Beijing Daxue Xuebao, Ziran Kexueban (1999), 35(4), 487-495

CODEN: PCTHAP; ISSN: 0479-8023

PB Beijing Daxue Chubanshe

DT Journal

LA Chinese

AB A novel plasminogen activator was constructed using scFv SZ51 as targeted mol., and **scu-PA-32k** as effect mol. SZ51 was a monoclonal antibody of GMP140 on activated human platelets. Polymerase chain reaction (PCR) was used to amplify the region of VK and VH from Fab of SZ51, and **scu-PA-32k**(leu144-leu411) from **urokinase** gene, resp. These fragments were joined together and inserted into the expression vector, pET-5a, via a NdeI site. After transforming into **E. coli BL21 (DE3)** plyS and inducing with IPTG, the **recombinant** protein was expressed in inclusion bodies. Western-Blotting showed that the protein could interact weakly with the multiple clonal antibody of **urokinase** in 8 M Urea. After renaturation and partial purification, the product had a strong fibrinolytic activity through activating plasminogen on fibrin plate, the specific activity was about 17,500 IU/mg, which showed the **recombinant** protein retained the activity of u-PA. The yield was almost 1.5 mg/100g wet bacteria.

L98 ANSWER 21 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1999:587341 HCAPLUS

DN 132:147326

TI Oxygen-regulated expression of heterologous gene in **Escherichia coli**

AU Tong, Qin; Yang, Shengli; Gong, Yi

CS Shanghai Research Center of Biotechnology, The Chinese Academy of Sciences, Shanghai, 200233, Peop. Rep. China

SO Shengwu Gongcheng Xuebao (1999), 15(3), 322-326

CODEN: SGXUED; ISSN: 1000-3061

PB Kexue Chubanshe

DT Journal

LA Chinese

AB The expression of Vitreosilla Hb gene (vgb) is regulated by the dissolved oxygen consistence in **E. coli**. A new system for expressing heterologous gene in **E. coli** regulated by dissolved oxygen consistence was constructed. It includes a host bacteria GJ100, which contains T7 RNA polymerase gene controlled by vgb promoter, and an expression vector on which the heterologous gene is under the control of T7 promoter. The results indicated that **E. coli** thioredoxin A, IgG binding domain of Staphylococcus protein A (ZZ), snake neurotoxin, salmon calcitonin hexa-polymer, human interleukin II (IL2) and human **pro-urokinase** genes could be expressed efficiently. The expression level of all genes is more than 30% of total cellular protein.

- IT **82657-92-9P, Prourokinase**  
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)  
(heterologous expression of; oxygen-regulated expression of heterologous gene in *Escherichia coli*)
- L98 ANSWER 22 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 1999:575396 HCAPLUS  
DN 131:282177  
TI Construction and expression of a novel **chimeric** protein consisting the A chain of tissue-type plasminogen activator and the **B chain of pro-urokinase**  
AU Zhao, Chunmei; Zhang, Hongtao; Hu, Meihao  
CS National Laboratory of Protein Engineering and Plant Genetic Engineering, Peking University, Beijing, 100871, Peop. Rep. China  
SO Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (1999), 15(4), 528-531  
CODEN: ZSHXF2; ISSN: 1007-7626  
PB Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui  
DT Journal  
LA Chinese  
AB A hybrid cDNA tu-pa, which contained Ser1-Thr263 of tissue-type plasminogen activator (t-PA) and Ser138-Leu411 of **pro-urokinase (pro-UK)** was constructed via **recombinant DNA** technol. and site-directed **mutagenesis**. And it was expressed in the baculovirus system. The activity on fibrin plate of the cell culture was 500 IU/mL. The cell culture was purified by immunoaffinity chromatog. SDS-PAGE and Western-blot showed that the mol. weight of tu-PA was about 60,000. The specific activity of tu-PA was 200,000 IU/mg protein. The fibrin affinity specificity of tu-PA was much higher than that of **pro-UK**.
- IT **82657-92-9P, Prourokinase**  
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)  
(expression of a novel **chimeric** protein consisting of A chain of tissue-type plasminogen activator and **B chain of pro-urokinase**)
- L98 ANSWER 23 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 1999:185499 HCAPLUS  
DN 131:14629  
TI High level expression of the deleted and **mutated urokinase**-single chain antibody Fv fusion gene in **Escherichia coli**  
AU Wang, Xiang; Yu, Weiyuan  
CS Institute of Biotechnology, Academy of Military Medical Sciences, Beijing, 100071, Peop. Rep. China  
SO Shengwu Gongcheng Xuebao (1999), 15(1), 23-27  
CODEN: SGXUED; ISSN: 1000-3061  
PB Kexue Chubanshe  
DT Journal  
LA Chinese  
AB The fusion gene of a specific anti-human fibrinogen D-dimer single chain antibody (scFv) and low mol. weight **single chain urokinase (scu-PA-32K)** was restricted, spliced and digested by exonuclease Bal31 to obtain a series of deletion **mutants**, and their expression in **E. coli** revealed that the key sequence which reduced its expression level resides in the range from 841 bp to 851 bp, in which tandem AGG codons (encoding arginine, rarely used in **E. coli**) exist. By PCR mediated site-**mutation**, we altered two AGG codons to CGT codons, and the **mutant** was more efficiently translated in **E. coli**; the expression level turned out to be about 30% of the total

bacterial proteins while that of the non-mutated nature gene was 2-3%.

L98 ANSWER 24 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
 AN 1998:757111 HCAPLUS  
 DN 130:105814  
 TI Fusion expression of human **pro-urokinase** with **E. coli** thioredoxin  
 AU Sun, Ai-Long; Hua, Zi-Chun; Yao, Jun; Yang, Yong-Hua; Yin, Da-Qiang  
 CS Pharmaceutical Biotechnology Key Laboratory, Department of Biochemistry, Nanjing University, Nanjing, 210093, Peop. Rep. China  
 SO Biochemistry and Molecular Biology International (1998), 46(3), 479-486  
 CODEN: BMBIES; ISSN: 1039-9712  
 PB Academic Press  
 DT Journal  
 LA English  
 AB Human **pro-urokinase** (**pro-UK**) was cloned into plasmid pET32b and fused to the **E. coli** thioredoxin (**trxA**). When expressed in **E. coli** AD494(**DE3**), the fusion protein **Trx-pro-UK** accumulated as insol. inclusion bodies and amounted to 35% of total cellular proteins. When co-expressed with mol. chaperones human protein disulfide isomerase (**PDI**) and **E. coli** GroESL, all the expressed products still existed in the form of insol. inclusion bodies. (c) 1998 Academic Press.  
 IT **82657-92-9P, Pro-urokinase**  
 RL: BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation); PROC (Process)  
 (human; fusion expression of human **pro-urokinase** with **E. coli** thioredoxin)

## RETABLE

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	Referenced File
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Caspers, P	1994	40	635	Cell Mol Biol	HCAPLUS
Collen, D	1980	43	77	Thromb Haemost	HCAPLUS
Dale, G	1994	7	925	Protein Eng	HCAPLUS
Derman, A	1993	262	1744	Science	HCAPLUS
Golobinoff, P	1989	337	44	Nature	
Hayer-Hartl, M	1995	269	836	Science	HCAPLUS
Hibino, Y	1988	52	329	Agric Biol Chem	HCAPLUS
Hiramatsu, R	1991	99	235	Gene	HCAPLUS
Holmes, W	1985	3	923	Bio/Technology	HCAPLUS
Hua, Z	1997	220	131	Biochem Biophys Res	
Hua, Z	1994	33	1215	Biochem Mol Biol Int	HCAPLUS
Hua, Z	1996	39	1093	Biochem Mol Biol Int	HCAPLUS
Humphreys, D	1995	270	28210	J Biol Chem	HCAPLUS
LaVallie, E		11	187	Bio/Technology	HCAPLUS
Laemmli, U	1970	227	680	Nature	HCAPLUS
Laminet, A	1990	9	2315	EMBO J	HCAPLUS
Maniatis, T	1989			Molecular Cloning: A	
Melnick, L	1990	265	801	J Biol Chem	HCAPLUS
Mizobata, T	1992	267	17773	J Biol Chem	HCAPLUS
Nelles, L	1987	262	5682	J Biol Chem	HCAPLUS
Orsini, G	1991	195	691	Eur J Biochem	HCAPLUS
Ostermeier, M	1996	271	10616	J Biol Chem	HCAPLUS
Ploug, J	1957	24	278	Biochim Biophys Acta	HCAPLUS
Puig, A	1994	269	7764	J Biol Chem	HCAPLUS
Roman, L	1995	92	8428	Proc Natl Acad Sci U	HCAPLUS
Surek, B	1991	32	388	Applied Microbiology	

Winkler, M	1985	3	990	Bio/Technology	HCAPLUS
Winkler, M	1985	25	4041	Biochemistry	
Wun, T	1982	257	7262	J Biol Chem	HCAPLUS

L98 ANSWER 25 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1998:631425 HCAPLUS

DN 129:240872

TI **Escherichia coli** protease triple deletion

**mutant** as host cell for stable expression of normally unstable proteins

IN Kanemori, Masaaki; Yanagi, Hideki; Yura, Takashi

PA HSP Research Institute, Inc., Japan

SO Eur. Pat. Appl., 14 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 866132	A2	19980923	EP 1998-104907	19980318 <--
	EP 866132	A3	19991208		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 10313863	A2	19981202	JP 1998-31718	19980213 <--
	JP 3325512	B2	20020917		
	CA 2226399	AA	19980919	CA 1998-2226399	19980316 <--
PRAI	JP 1997-85914	A	19970319		<--

AB The **Escherichia coli** mutant carrying a triple deletion **mutation** in the hslV/U gene, the clpPX gene, and lon gene, and possessing a function to stabilize an unstable protein expressed in **Escherichia coli**, a method for preparing the **Escherichia coli** mutant, a method for stably expressing an unstable protein in **Escherichia coli** by using the **Escherichia coli** mutant, a method for stabilizing an unstable introducing an expression vector carrying a gene encoding a foreign protein into the **Escherichia coli** mutant, and a method for preparing a foreign protein using the transformant. Thus, the **E. coli** mutant strain containing the triple deletion **mutation** is useful for stable expression of normally unstable proteins such as  $\sigma$ 32, a pollen antigen Cryj2, and human **prourokinase**.

IT 82657-92-9, **Prourokinase**

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)

(stable expression of; **Escherichia coli** protease triple deletion **mutant** as host cell for stable expression of normally unstable proteins)

L98 ANSWER 26 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1998:415434 HCAPLUS

DN 129:226262

TI High-level expression in **Escherichia coli** and purification of human **pro-urokinase** cDNA

AU Peng, Guihong; Ma, Zhong; Xue, Yuming; Chen, Yuhong; Zhu, Dexu

CS Department of Biochemistry, National Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, Peop. Rep. China

SO Shengwu Gongcheng Xuebao (1997), 13(4), 362-367

CODEN: SGXUED; ISSN: 1000-3061

PB Kexue Chubanshe

DT Journal

LA Chinese

AB A chemical synthesized human **pro-urokinase** (**pro-UK**) cDNA was cloned into the expression vector pET-11d, and

expressed in *E. coli* BL21(DE3) pLysS under the control of T7 promoter. The expression level of the recombinant pro-UK was over 15% of total bacterial proteins as inclusion bodies. The specific activity of the purified human pro-UK was about 110000 IU/mg.

IT 82657-92-9P, Pro-urokinase

RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)

(expression in *Escherichia coli* and purification of human pro-urokinase cDNA)

L98 ANSWER 27 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1998:261401 HCAPLUS

DN 129:92056

TI Construction of urokinase mutant Glu154-mtcu-PA and characterization of its properties

AU Peng, Guihong; Ma, Zhong; Xue, Yuming; Chen, Yuhong; Zhu, Dexu

CS Department of Biochemistry and State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, Peop. Rep. China

SO Shengwu Huaxue Yu Shengwu Wuli Xuebao (1997), 29(6), 547-552

CODEN: SHWPAU; ISSN: 0582-9879

PB Shanghai Kexue Jishu Chubanshe

DT Journal

LA Chinese

AB The recombinant single chain

urokinase-type plasminogen activator (rscu-PA) and a mutant constructed by in vitro site-specific mutagenesis of Arg154 in rscu-PA to Glu154 (Glu154-mscu-PA) were both expressed in *E. coli*. The expressed products were both purified to homogeneity by in vitro denaturation and renaturation with Zn<sup>2+</sup> selective precipitation and immuno-affinity chromatog. The plasmin sensitivity assay indicated that the activation of this single chain Glu154-mscu-PA by plasmin was essentially identical to that of rscu-PA. After activation by plasmin, the kinetic consts. against synthetic substrate S2444 of the resulted 2 chain form of Glu154-mscu-PA (Glu154-mtcu-PA) and that of rscu-PA (rtcu-PA) were 87 and 80  $\mu$ M, resp. It indicate that the catalytic active site of the Glu154-mtcu-PA is not changed by the mutation. Yet both 125I-fibrin plasma-clot lysis and fibrinogenolysis in plasma showed that the Glu154-mtcu-PA possessed a better affinity and selectivity for fibrin than rtcu-PA or even better than rscu-PA.

L98 ANSWER 28 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1997:637172 HCAPLUS

DN 127:328291

TI Identification of a flexible loop region (297-313) of urokinase-type plasminogen activator, which helps determine its catalytic activity

AU Sun, Ziyong; Jiang, Yongping; Ma, Zong; Wu, Hui; Liu, Bei-Fang; Xu, Yuming; Tang, Wei; Cheno, Yuhong; Li, Cuizhen; Zhu, Dexu; Gurewich, Victor; Liu, Jian-Ning

CS Vascular Research Laboratory, Institute for Prevention of Cardiovascular Disease, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, 02215, USA

SO Journal of Biological Chemistry (1997), 272(38), 23818-23823

CODEN: JBCHA3; ISSN: 0021-9258

PB American Society for Biochemistry and Molecular Biology

DT Journal

LA English

AB Prourokinase has much higher intrinsic catalytic activity than other zymogens of the serine protease family. Lys300(c143)

("cnnn" next to the position nos. of residues indicates chymotrypsin numbering) in an apparent "flexible loop" region

(297-313) was previously shown to be an important determinant of this intrinsic catalytic activity. This was related to the **loop** allowing the pos. charge of **Lys300(c143)** to transiently interact with **Asp355(c194)**, thereby inducing an active conformation of the protease domain. To further test this hypothesis, the charge at position 300(c143) and the **flexibility** of the **loop** were altered using site-directed **mutagenesis** designed according to a computer model to affect the interaction between **Lys300(c143)** and **Asp355(c194)**. When the charge at **Lys300(c143)** but not **Lys313(c156)** was reduced, a significant reduction in the intrinsic catalytic activity occurred. Similarly, when the **flexibility (wobbliness)** of the **loop** was enhanced reducing the size of side-chain, the intrinsic catalytic activity was also reduced. By contrast, when the **loop** was made less **flexible**, the intrinsic catalytic activity was increased. These findings were consistent with the hypothesis. The effects of these **mutations** on 2-chain activity were less and often discordant with the intrinsic catalytic activity, indicating that they can be modulated independently. This structure-function disparity can be exploited to create a more zymogenic **prourokinase** (lower intrinsic catalytic activity) with a high catalytic activity, as exemplified by 2 of the **mutants**. The changes in intrinsic catalytic activity and 2-chain activity induced by the **mutations** were due to changes in *k<sub>cat</sub>* rather than *K<sub>m</sub>*. Some significant structure-function differences between **prourokinase** and its highly homologous counterpart, tissue plasminogen activator, were also found.

# IT 82657-92-9, Prourokinase

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
(identification of a **flexible loop** region (297-313)  
in human **urokinase**-type plasminogen activator which helps  
determine its catalytic activity)

## RETABLE

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	Referenced File
=====	=====	=====	=====	=====	=====
Bugge, T	1996	87	709	Cell	HCAPLUS
Gurewich, V	1989	82	1956	J Clin Invest	
Huber, A	1978	11	114	Acc Chem Res	
Lamba, D	1996	258	117	J Mol Biol	HCAPLUS
Lijnen, H	1990	265	5232	J Biol Chem	HCAPLUS
Liu, J	1992	31	6311	Biochemistry	HCAPLUS
Liu, J	1996	35	14070	Biochemistry	HCAPLUS
Liu, J	1993	81	980	Blood	HCAPLUS
Liu, J	1992	267	15289	J Biol Chem	HCAPLUS
Liu, J	1995	270	8408	J Biol Chem	HCAPLUS
Liu, J	1991	88	2012	J Clin Invest	HCAPLUS
Madison, E	1993	262	419	Science	HCAPLUS
Moscatelli, D	1988	948	67	Biochim Biophys Acta	HCAPLUS
Orini, G	1991	195	691	Eur J Biochem	
Pannell, R	1986	67	1215	Blood	HCAPLUS
Pannell, R	1987	69	22	Blood	HCAPLUS
Pannell, R	1988	81	853	J Clin Invest	HCAPLUS
Petersen, L	1990	29	3451	Biochemistry	HCAPLUS
Petersen, L	1988	263	11189	J Biol Chem	HCAPLUS
Rijken, D	1982	257	2920	J Biol Chem	HCAPLUS
Romer, J	1994	102	519	J Invest Dermatol	MEDLINE
Sappino, A	1989	109	2471	J Cell Biol	HCAPLUS
Spraggon, G	1995	3	681	Structure	HCAPLUS
Strickland, S	1976	9	231	Cell	HCAPLUS
Tachias, K	1996	271	28749	J Biol Chem	HCAPLUS
Tachias, K	1997	272	28	J Biol Chem	HCAPLUS
Tate, K	1986	26	338	Biochemistry	

Valinsky, J	1981	25	471	Cell	HCAPLUS
Voskuilen, M	1987	262	5944	J Biol Chem	HCAPLUS
Winkler, M	1986	25	4041	Biochemistry	HCAPLUS

L98 ANSWER 29 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1997:400620 HCAPLUS

DN 127:132590

TI Dissection of intrinsic catalytic activity of **pro-urokinase**

AU Wu, H.; Sun, Z. Y.; Liu, B. F.; Ma, Z.; Xue, Y. M.; Tang, W.; Jiang, Y. P.; Gurewich, V.; Zhu, D. X.; Liu, J. N.

CS Vascular Research Laboratory, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, 02215, USA

SO Protein Engineering (1997), 10(Suppl.), 70

CODEN: PRENE9; ISSN: 0269-2139

PB Oxford University Press

DT Journal

LA English

AB Site-directed **mutagenesis** of **prourokinase** proved **Lys300** is a key structural determinant in its activity. The **flexible** nature of the **loop** made up of amino acids 297-313 allowed **Lys300** to form a salt bridge with Asp335 which generated most of the intrinsic catalytic activity.

IT 82657-92-9, **Prourokinase**

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)

(site-directed **mutagenesis** of **prourokinase**

catalytic activity is generated by **Lys300** forming a salt bridge with Asp335)

RETABLE

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	Referenced File
Liu, J	1996			Biochemistry	
Madoson, E	1993	262	419	Science	

L98 ANSWER 30 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1997:316029 HCAPLUS

DN 127:46884

TI **Mutation** of Arg154 to Gly154 in **urokinase** augments its fibrin-specificity

AU Peng, Guihong; Ma, Zhong; Kuai, Letian; Zhu, Dexu

CS Dep. Biochem. and Natl. Lab. Pharmaceutical Biotechnology, Nanjing Univ., Nanjing, 210093, Peop. Rep. China

SO Biochemistry and Molecular Biology International (1997), 41(5), 887-894

CODEN: BMBIES; ISSN: 1039-9712

PB Academic

DT Journal

LA English

AB **Recombinant single-chain urokinase**

-type plasminogen activator (rscu-PA) and its **mutant** constructed by in vitro site specific **mutagenesis** of Arg154 in rscu-PA to Gly154 (mscu-PA) were both expressed in **Escherichia coli**

. After in vitro denaturation and renaturation, the rscu-PA and mscu-PA were purified to homogeneity by Zn<sup>2+</sup> selective precipitation, anti-u-PA IgG-sepharose CL4B affinity chromatog. After activation by plasmin, the kinetic consts. for the resultant mtcu-PA against synthetic substrate S2444 hydrolysis were found to be essentially identical to **recombinant 2-chain urokinase**-type plasminogen activator (rtcu-PA), suggesting that no impairment had been exerted on the catalytic active site of mtcu-PA. However, both 125I-fibrin plasma-clot lysis and fibrinogenolysis showed that mtcu-PA possessed a higher fibrinolytic

activity but hardly any degradation of fibrinogen in plasma compared to rtcu-PA and rscu-PA. Thus, the substitution of Arg154 by Gly154 in tcu-PA promoted the fibrin-specificity of **urokinase**.

## RETABLE

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	Referenced File
Bennett, J	1982	257	8049	J Biol Chem	HCAPLUS
Bradford, M	1976	72	248	Anal Biochem	HCAPLUS
Graffney, J	1990	64	398	Health Thromb Haemos	
Gurewich, V	1992	667	224	Annals of the New Yo	MEDLINE
Husain, S	1993	268	8575	J Biol Chem	
John, R	1990	27	6374	Biochemistry	
Laemmli, U	1970	227	680	Nature	HCAPLUS
Lijnen, H	1987		359	Eur J Biochem	HCAPLUS
Lijnen, H	1986	261	1253	J Biol Chem	HCAPLUS
Liu, J	1993	81	980	Blood	HCAPLUS
Liu, J	1991	10	1035	Science in China (se	
Ma, Z	1996	39	523	Science in China (ser	HCAPLUS
Nelles, L	1987	262	5682	J Biol Chem	HCAPLUS
Ploug, T	1957	24	278	Biochem Biophys Acta	
Rampling, M	1976	678	43	Clin chem Acta	
Sambrook, J	1989			Molecular cloning, A	
Song, A	1992	35	966	Science in China Ser	HCAPLUS
Stump, D	1986	261	17120	J Biol Chem	HCAPLUS
Valery, N	1995	270	8680	J Biol Chem	
Verstraete, M	1986	67	1529	Blood	HCAPLUS
Wrinkler, M	1986	25	4041	Biochemistry	

L98 ANSWER 31 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1996:726431 HCAPLUS

DN 126:3632

TI **Urokinase mutant** with better fibrin-specificity

AU Ma, Zhong; Yu, Ruihong; Hua, Zichun; Zhu, Dexu

CS Department Biochemistry, Nanjing University, Nanjing, 210093, Peop. Rep. China

SO Science in China, Series C: Life Sciences (1996), 39(5), 523-533

CODEN: SCCLFO; ISSN: 1006-9305

PB Science in China Press

DT Journal

LA English

AB A 150-156 amino acid-deleted **single-chain**

**urokinase**-type plasminogen activator (dscu-PA) and its **recombinant** wild-type counterpart (rscu-PA) were both expressed in **Escherichia coli**. After denaturation and renaturation in vitro, the expressed products were both purified to a single silver-stained band by means of IgG affinity chromatog. After activation by plasmin, similar enzymic consts. based on the hydrolysis of synthetic substrate S2444 by the two-chain mol. forms of dscu-PA and rscu-PA, or native tcu-PA were observed, suggesting that no impairment had been exerted on the catalytic active site of dtcu-PA by the 150-156 amino acids deletion. In both in vitro fibrin-clot and 125I-fibrin sepharose lysis tests, dtcu-PA showed a significantly higher fibrinolytic activity than rtcu-PA or rscu-PA. Hardly any effect on the concentration of fibrinogen in plasma was found in dtcu-PA. It was concluded that dtcu-PA had a higher fibrin specificity and that tcu-PA could be provided with better fibrin specificity by means of **mutation**.

IT 82657-92-9, **Single-chain urokinase**

-type plasminogen activator

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)

(development of **urokinase**-type plasminogen activator **mutant** with better fibrin-specificity)

L98 ANSWER 32 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1996:632233 HCAPLUS

DN 125:295880

TI A site-directed **mutagenesis** of **pro-urokinase**

which substantially reduces its intrinsic activity

AU Liu, Jian-Ning; Tang, Wei; Sun, Zi-Yong; Kung, Wendy; Pannell, Ralph;

**Sarmientos, Paolo**; Gurewich, Victor

CS Institute for the Prevention of Cardiovascular Disease, Harvard Medical School, Boston, MA, 02215, USA

SO Biochemistry (1996), 35(45), 14070-14076

CODEN: BICHAW; ISSN: 0006-2960

PB American Chemical Society

DT Journal

LA English

AB **Single-chain urokinase-type plasminogen**

activator or **pro-urokinase** is a zymogen with an intrinsic catalytic activity which is greater than that of most other zymogens. To study the structural basis for this activity, a three-dimensional homol. model was calculated using the crystallog. structure of chymotrypsinogen, and the structure-function relationship was studied using site-directed **mutagenesis** and kinetic anal. This model

revealed a unique **Lys300** in **pro-urokinase**

which could form a weak interaction with Asp355, adjacent to the active site Ser356. It was postulated that this **lysine**, by its

$\epsilon$ -amino group, may serve to pull Ser356 close to the active

position, thereby inducing the higher intrinsic activity of **pro-**

**urokinase**. This was consistent with the published finding that a

homologous **lysine** (Lys416) in single chain tissue plasminogen

activator when **mutated** to serine induced some reduction in activity.

To test this hypothesis, a site-directed **mutant** with a neutral

residue (**Lys300**  $\rightarrow$  Ala) was produced and characterized.

The Ala300-**pro-urokinase** had a 40-fold lower

amidolytic activity than that of **pro-urokinase**. It

was also stable in plasma at much higher concns. than **pro-**

**urokinase**, reflecting much attenuated plasminogen activation.

Plasmin activatability was comparable to that of **pro-**

**urokinase**, but the resultant two-chain derivative (Ala300-

**urokinase**) had a lower enzymic activity ( $\approx 33\%$  that of

**urokinase**) due to a reduction of  $k_{cat}$ . Interestingly, the  $K_M$  of

two-chain Ala300-**urokinase** against plasminogen was 5.8-fold

lower than that of **urokinase**, being similar to that of

**pro-urokinase** which has a  $K_M$  about 5-fold lower than

**urokinase**. In conclusion, the hypothesis that **Lys300** is

a key structural determinant of the high intrinsic activity of **pro**

-**urokinase** was confirmed by these studies. This residue also

appears to be important for the full expression of the enzymic activity of **urokinase**.

IT 56-87-1, **Lysine**, biological studies

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)

(position 300; site-Directed **Mutagenesis** of **Pro-**

**urokinase** which Substantially Reduces Its Intrinsic Activity)

IT 82657-92-9, **Single-chain urokinase**

-type plasminogen activator

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)

(site-Directed **Mutagenesis** of **Pro-urokinase**

which Substantially Reduces Its Intrinsic Activity)

L98 ANSWER 33 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1996:392791 HCAPLUS

DN 125:106288

- TI High expression of human **pro-urokinase** promoted by  
T7 promoter in **Escherichia coli**
- AU Dong, Chen; Chen, Xuaochun; Chen, Yuhong; Ma, Zhong; Xia, Yan; Hua, Zichun
- CS Department of Biochemistry, Nanjing University, Nanjing, 210093, Peop.  
Rep. China
- SO Nanjing Daxue Xuebao, Ziran Kexue (1995), 31(4), 606-610  
CODEN: NCHPAZ; ISSN: 0469-5097
- PB Nanjing Daxue
- DT Journal
- LA Chinese
- AB **Pro-urokinase (Pro-UK)**, the second generation of thrombolytic agent, was superior to **urokinase** by its more specific affinity to fibrin and mild enzymic activity, which meant that it had less tendency to cause hemorrhage when injected in a large dose. But because of its rare natural sources, insufficient **Pro-UK** would be available for clin. therapy unless it was produced by genetic engineering methods. Up to now, the expression level of **Pro-UK** cDNA in **E. coli** has hardly been more than 2% of total bacterial proteins. In this article, a human **Pro-UK** cDNA controlled by T7 promoter was reported to be successfully expressed in **E. coli** with 18% expression level. To create pET3d/**Pro-UK** cDNA, a fragment of **Pro-UK** gene cleaved from pUC9/**Pro-UK** plasmid by HindIII (Klenow blunted) and BspHI, was inserted into the pET3d expression vector, which was cut with BamHI (Klenow blunted) and NcoI. The pos. **recombined** plasmid was confirmed by cutting with several restriction enzymes, and was transformed into **E. coli** BL21(DE3) Lyss. Following induction by 1 mmol/L IPTG, **Pro-UK** cDNA was expressed with an extra band of 43 kDa on SDS-PAGE, and the band was proved to be human **Pro-UK** by western blotting anal. Densitometric scanning revealed that the expression level of **Pro-UK** was 18% of total cellular protein. The expression bacteria were sonicated and the resultant lysate and sediment were collected sep. After denaturation and renaturation in vitro, the sediment part displayed fibrinolytic activity of about 300,000 IU/L culture medium, whereas the supernatant rarely had the same activity. This suggests that the expression product of pET3d/**Pro-UK** was mainly in the form of inclusion bodies.
- IT **82657-92-9P, Prourokinase**  
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)  
(high expression of **recombinant** human **prourokinase** promoted by T7 promoter in **Escherichia coli**)
- L98 ANSWER 34 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
- AN 1996:176193 HCAPLUS
- DN 124:230074
- TI Renaturation of **recombinant** human **pro-urokinase** expressed in **Escherichia coli**
- AU Hua, Zi-Chun; Dong, Chen; Zhu, De-Xu
- CS Pharmaceutic Biotechnology Key Lab., Nanjing Univ., Nanjing, 210093, Peop.  
Rep. China
- SO Biochemical and Biophysical Research Communications (1996),  
220(1), 131-6  
CODEN: BBRCA9; ISSN: 0006-291X
- PB Academic
- DT Journal
- LA English
- AB A synthetic gene encoding human **pro-urokinase** (**pro-UK**) with **E. coli**-favored codon usage was cloned into plasmid pET-3d and expressed in **E.**

**coli BL21(DE3)** LyssS strain. The expressed products, which accumulated as inactive inclusion bodies, were denatured and renatured in vitro. A broad range of parameters such as pH, protein concentration, denaturant concentration, the use of cosolvent polyethylene glycol and presence of basic or acidic amino acid was examined. At optimal renaturation condition, **pro-UK** activity of more than 1000 I.U. was obtained from 1 mL cell culture.

IT **82657-92-9P, Pro-urokinase**  
 RL: BMF (Bioindustrial manufacture); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation); PROC (Process)  
 (renaturation of **recombinant** human **pro-urokinase** expressed in **Escherichia coli**)

L98 ANSWER 35 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1995:796989 HCAPLUS

DN 123:224082

TI Native and non-glycosylated **recombinant single-chain urokinase**-type plasminogen activator are recognized by different receptor systems on rat parenchymal liver cells

AU van der Kaaden, Marieke E.; Rijken, Dingeman C.; Groeneveld, Eleonore; van Berkel, Theo J. C.; Kuiper, Johan

CS Sylvius Lab., Univ. Leiden, Leiden, Neth.

SO Thrombosis and Haemostasis (1995), 74(2), 722-9

CODEN: THHADQ; ISSN: 0340-6245

PB Schattauer

DT Journal

LA English

AB The recognition systems mediated the clearance of glycosylated high-mol.-weight **single-chain urokinase**-type plasminogen activator (HMW-**scu-PA**, produced in human embryonic kidney cells) and **recombinant** non-glycosylated **scu-PA** (rscu-PA, produced in **E. coli**) were analyzed by studying their binding characteristics to freshly isolated rat parenchymal liver cells. The binding of 125I-HMW-**scu-PA** at 4° was calcium-dependent and of high affinity ( $K_d$  - 37.6 nM) and could be inhibited by low mol. weight two-chain u-PA (LMW-tcu-PA) and lactose, but not by the low d. lipoprotein receptor-related protein (LRP)-associated 39-kDa protein (RAP), rscu-PA or a **mutant** form lacking amino acids 11-135 (Delta 125-rscu-PA). Removal of the carbohydrate side chain of HMW-**scu-PA** by treatment with N-glycosidase F, completely reduced the specific binding to the parenchymal cells and strongly reduced its competition with 125I-HMW-**scu-PA** in cell binding. **Recombinant scu-PA** also bound with high affinity ( $K_d$  = 38.7 nM) to the parenchymal liver cells. The binding of 125I-rscu-PA could be competed for by unlabeled rscu-PA while Delta 125 125-rscu-PA, LMW-tcu-PA or lactose were ineffective. In contrast to HMW-**scu-PA**, binding of 125I-rscu-PA could be effectively inhibited by RAP ( $K_i$  = 1.1 nM), while also its association and degradation, as determined at 37°, were inhibited by RAP. Pretreatment of the parenchymal cells with proteinase K supplied further evidence for the involvement of two different receptor systems. The binding of rscu-PA was decreased for 91%, while that of HMW-**scu-PA** showed a decrease of 51%. It is suggested that native HMW-**scu-PA** is bound and degraded by the rat parenchymal liver cells via a lectin-like recognition site, while non-glycosylated **recombinant scu-PA** is bound and degraded by rat parenchymal liver cells via the low d. lipoprotein receptor-related protein (LRP). The differences in recognition system for native and **recombinant** proteins by liver cells suggest that the glycosylation of **recombinant** proteins, as obtained in mammalian expression systems, can be important for their physiol. fate and their

- pharmacol. application.
- IT **82657-92-9, Single-chain urokinase**  
-type plasminogen activator **82657-92-9D, Single-chain urokinase**-type plasminogen activator, nonglycosylated  
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
(native and nonglycosylated **recombinant single-chain urokinase**-type plasminogen activator are recognized by different receptor systems on rat parenchymal liver cells)
- L98 ANSWER 36 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 1995:419764 HCAPLUS  
DN 123:2186  
TI Use of bacteriophage T7 RNA polymerase to direct expression of cloned **pro-urokinase** gene in **Escherichia coli**  
AU Sui, Guangchao; Liu, Fang; Hu, Meihao  
CS College Life Sciences, Peking University, Beijing, 100871, Peop. Rep. China  
SO Beijing Daxue Xuebao, Ziran Kexueban (1994), 30(6), 728-33  
CODEN: PCTHAP; ISSN: 0479-8023  
PB Beijing Daxue Chubanshe  
DT Journal  
LA Chinese  
AB Human **pro-urokinase (pro-UK)** was produced in **Escherichia coli**. This was done using the T7 gene .vphi.10 promoter on the vector pET3c. After induction with IPTG, the **pro-UK** was expressed with an expression level of about 1600 IU/L of cell culture assayed on a fibrin plate after a denaturation-refolding procedure. The product was characterized by Western blot anal. The blot displayed a major band at 43 kDa slightly lower than natural 54 kDa **pro-UK**, probably due to the lack of glycosylation of **pro-UK** in **E. coli**.
- IT **82657-92-9P, Prourokinase**  
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)  
(use of bacteriophage T7 RNA polymerase to direct expression of cloned **pro-urokinase** gene in **Escherichia coli**)
- L98 ANSWER 37 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 1994:571656 HCAPLUS  
DN 121:171656  
TI Expression of a fibrinolytically active human **pro-urokinase** fusion protein in **Escherichia coli**  
AU Hua, Zichun; Jie, Li; Zhu, Dexu  
CS Department Biochemistry, Nanjing University, Nanjing, 210008, Peop. Rep. China  
SO Biochemistry and Molecular Biology International (1994), 33(6), 1215-20  
CODEN: BMBIES; ISSN: 1039-9712  
DT Journal  
LA English  
AB The gene encoding human **pro-urokinase (pro-UK)** was cloned into plasmid pEZZ318 and fused to the gene coding for the signal peptide of staphylococcal protein A and IgG binding domain. The fusion protein which was synthesized under the control of the T7 promoter in **Escherichia coli** and secreted into the growth medium was found to be fibrinolytically active. Approx. 60% of the total activity was secreted into the culture medium, where

levels of activity approached 150,000 I.U./L and about 40% of the total activity remained in the cell lysate with levels of activity around 100,000 I.U./L. The fusion protein was purified in a single step by IgG affinity chromatog. These results demonstrate that human **pro-UK** can be synthesized and secreted by *E. coli* as a fibrinolytically active fusion protein.

IT **82657-92-9, Pro-urokinase**

RL: BIOL (Biological study)

(gene for, of human, cloning of, in *Escherichia coli*)

L98 ANSWER 38 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1994:553198 HCAPLUS

DN 121:153198

TI A methylotrophic and glucotrophic **mutant** strain for producing a heterologous protein

IN Okabayashi, Ken; Ohmura, Takao; Yokoyama, Kazumasa; Kawabe, Haruhide

PA Green Cross Corp., Japan

SO Eur. Pat. Appl., 13 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 606917	A2	19940720	EP 1994-100460	19940113 <--
	EP 606917	A3	19950719		
	R: BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE				
	JP 06209763	A2	19940802	JP 1993-4289	19930113 <--
	US 5643792	A	19970701	US 1994-181242	19940113 <--
PRAI	JP 1993-4289	A	19930113	<--	

AB A methylotrophic and glucotrophic **mutant** strain and its use for producing a heterologous protein are disclosed. The **mutant** strain can be grown in a medium containing both methanol and glucose, with the effect that the growth of the strain and production of a heterologous protein proceed at the same time. Accordingly, a heterologous protein can be produced in a large amount in a short time. *Pichia pastoris* GCP104 (containing human serum albumin expression cassette under the control of AOX1 promoter) having AOX1 gene deleted was used to select strain GCP101 that exhibits **mutation** on the AOX2 promoter and, as a result, efficient utilization of MeOH. Strain GCP101 was then EMS-mutated to select **mutant** ECCR72, that was capable of utilizing both glucose and MeOH and produce an increased amount of HSA.

IT **82657-92-9P, Prourokinase**

RL: PREP (Preparation)

(manufacture of, with methylotrophic and glucotrophic **mutant**)

L98 ANSWER 39 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1994:100542 HCAPLUS

DN 120:100542

TI Protease-resistant **urokinases**, their preparation with **recombinant** cells, and their use in pharmaceuticals

IN Blaber, Michael; Heyneker, Herbert L.; Vehar, Gordon A.

PA Genentech, Inc., USA

SO U.S., 12 pp. Cont.-in-part of U.S. Ser. No. 725,468, abandoned.

CODEN: USXXAM

DT Patent

LA English

FAN.CNT 5

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5219569	A	19930615	US 1985-766858	19850816 <--
	DK 8601813	A	19861023	DK 1986-1813	19860421 <--

DK 175304	B1	20040816		
EP 200451	A1	19861105	EP 1986-302981	19860421 <--
EP 200451	B1	19931215		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL				
HU 40696	A2	19870128	HU 1986-1656	19860421 <--
HU 202586	B	19910328		
DD 251791	A5	19871125	DD 1986-289442	19860421 <--
ZA 8602976	A	19871230	ZA 1986-2976	19860421 <--
DD 258026	A5	19880706	DD 1986-289443	19860421 <--
DD 264939	A5	19890215	DD 1986-310554	19860421 <--
AT 68825	E	19911115	AT 1986-302980	19860421 <--
AT 98686	E	19940115	AT 1986-302981	19860421 <--
ES 554249	A1	19871116	ES 1986-554249	19860422 <--
US 5073494	A	19911217	US 1990-522480	19900511 <--
US 5147643	A	19920915	US 1991-741120	19910805 <--
US 5756093	A	19980526	US 1994-275335	19940714 <--
US 5714372	A	19980203	US 1994-306928	19940915 <--
PRAI US 1985-725468	B2	19850422	<--	
US 1985-766858	A	19850816	<--	
US 1986-846697	A	19860401	<--	
EP 1986-302980	A	19860421	<--	
EP 1986-302981	A	19860421	<--	
US 1987-71506	B1	19870709	<--	
US 1988-186494	B1	19880426	<--	
US 1990-522480	A3	19900511	<--	
US 1991-741120	A2	19910805	<--	
US 1991-808366	B1	19911216	<--	
US 1991-808537	B1	19911216	<--	
US 1993-101276	B1	19930802	<--	
US 1993-126114	B1	19930923	<--	

AB Single-chain human **urokinase** analogs modified at residues **Lys**-135 or -136 and/or at Arg-156 to **Lys**-158 are resistant to proteolytic cleavage. [.DELTA.**Lys**-136,Phe-157, **Lys**-158]human **urokinase** was produced with recombinant *E. coli*, purified, and tested for plasmin resistance. The analog retained most of the plasminogen activating capacity and was far less susceptible to plasmin activation than was the recombinant native single-chain human **urokinase**.

L98 ANSWER 40 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1993:554583 HCAPLUS

DN 119:154583

TI Implication of cysteine residues in the activity of **single-chain urokinase**-plasminogen activator

AU Hamelin, Jocelyne; Sarmientos, Paolo; Orsini, Gaetano; Galibert, Francis

CS Cent. Hayem, Hop. Saint-Louis, Paris, 75475, Fr.

SO Biochemical and Biophysical Research Communications (1993), 194(2), 978-85

CODEN: BBRCA9; ISSN: 0006-291X

DT Journal

LA English

AB **Single-chain urokinase**-type plasminogen

activator (I) contains 24 cysteine residues involved in 12 disulfide bonds and distributed all along the 3 domains of the protein. In order to investigate the role of these disulfide bridges in the catalytic activities of I, site-specific **mutagenesis** was used to construct 10 **mutants** in which some cysteine residues were changed to serine residues. Each **mutated** DNA fragment was cloned into a prokaryotic expression vector and the protein expressed in *E. coli*. **Mutant** proteins of the expected size were produced and analyzed for amidolytic and fibrinolytic activities. From

*Ab T7 shw  
delajeno n  
8/20/93*

this, it was shown that: (1) the disulfide bonds in the epidermal growth factor (EGF)-like and in the kringle domains are not necessary. Moreover, disulfide bond deletion in the kringle domain improved those catalytic activities; (2) on the contrary, the disulfide bridges in the catalytic domain are essential for maintaining both activities.

- L98 ANSWER 41 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 1993:447380 HCAPLUS  
DN 119:47380  
TI The **pro-urokinase** molecule: Structural and biotechnological aspects  
AU **Sarmientos, Paolo**; Lansen, Jacqueline; Mazue, Guy; Carminati, Paolo; Roncucci, Romeo  
CS Farmitalia Carlo Erba, Milan, Italy  
SO Biotec (Brescia) (1990), 5(2), 61-3, 65-7  
CODEN: BBRCED; ISSN: 0393-9146  
DT Journal; General Review  
LA English/Italian  
AB A review with no refs. of the authors' work on the structure, production, and potential as a thrombolytic agent of human **single-chain urokinase**-type plasminogen activator and a low-mol.-weight derivative obtained by site-directed **mutagenesis**.  
IT **82657-92-9**  
RL: BIOL (Biological study)  
(structural and biotechnol. aspects of)
- L98 ANSWER 42 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 1993:421552 HCAPLUS  
DN 119:21552  
TI High expression vectors for the production of **recombinant single-chain urinary plasminogen activator** from **Escherichia coli**  
AU Brigelius-Flohe, Regina; Steffens, Gerd; Strassburger, Wolfgang; Flohe, Leopold  
CS Cent. Res., Gruenenthal G.m.b.H., Aachen, W-5100, Germany  
SO Applied Microbiology and Biotechnology (1992), 36(5), 640-9  
CODEN: AMBIDG; ISSN: 0175-7598  
DT Journal  
LA English  
AB An expression cassette containing a synonymous gene for human **single-chain urokinase**-type plasminogen activator (Rscu-PA) 5'-flanked by a **trp promoter** and the **Shine-Dalgarno** sequence of the **xyl A operon** of *Bacillus subtilis* and terminated by the terminators **trp A** and **Tn10** was constructed and inserted into a pBR322 derivative to yield pBF160. When compared to pUK54 **trp 207-1** containing the natural scuPA gene without the **Shine-Dalgarno** sequence and terminator, the expression efficiency of pBF160 in **Escherichia coli** strains was improved by one order of magnitude. Replacement of the **trp** by the **tac promoter** (pBF171) did not affect expression. Inserting the **Shine-Dalgarno** sequence and **Tn10** terminator into pUK54 **trp 207-1** (pWH1320) slightly increased the expression level, whereas elimination of the **Shine-Dalgarno** sequence and the terminators from pBF160 with almost complete conservation of the synonymous structural gene (pBF191) significantly reduced the expression. Variation of the distance between the **Shine-Dalgarno** sequence and the start codon between 8 and 10 bp (pBF163) proved irrelevant. In conclusion, poor expression of mammalian genes in *E. coli* may result from both improperly designed regulatory elements and structural features of the coding region and therefore de-novo synthesis of the gene may be required to obtain satisfactory expression.  
IT **146481-75-6 146481-76-7**  
RL: PRP (Properties); BIOL (Biological study)

(nucleotide sequence of)

IT 146481-77-8

RL: PRP (Properties); BIOL (Biological study)

(nucleotide sequence of, complete)

L98 ANSWER 43 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1993:119887 HCAPLUS

DN 118:119887

TI Plasminogen activator analogs and their preparation using microorganisms

IN Steffens, Gerd J.; Guenzler, Wolfgang A.; Flohe, Leopold; Brigelius-Flohe, Regina E.; Wolf, Bernard

PA Gruenenthal GmbH, Germany

SO Ger. Offen., 33 pp.

CODEN: GWXXBX

DT Patent

LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 4101736	A1	19920723	DE 1991-4101736	19910122 <--
	RU 2108387	C1	19980410	RU 1992-5010518	19920109 <--
	HU 63877	A2	19931028	HU 1992-127	19920115 <--
	HU 212510	B	19960729		
	EP 496327	A1	19920729	EP 1992-100864	19920120 <--
	EP 496327	B1	20010816		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, PT, SE				
	AT 204326	E	20010915	AT 1992-100864	19920120 <--
	ES 2164048	T3	20020216	ES 1992-100864	19920120 <--
	PT 496327	T	20020228	PT 1992-100864	19920120 <--
	FI 9200263	A	19920723	FI 1992-263	19920121 <--
	JP 06169770	A2	19940621	JP 1992-8765	19920121 <--
	LT 3948	B	19960527	LT 1993-1532	19931206 <--
	HK 1010108	A1	20020404	HK 1998-110955	19980925 <--
PRAI	DE 1991-4101736	A	19910122	<--	

OS MARPAT 118:119887

AB Analogs of single-chain urinary plasminogen activator (**scu-PA**) with modified terminal regions (Ser-X1-X2-scu-PA143-403-Z-Leu-Ala-LeuOH (I) (X1 = Asn, Pro, Ser; X2 = bond, Glu, Pro-Glu, Pro-Pro-Glu, Glu-Leu-**His**-Leu-Leu-Gln-Val-Pro-Ser-Asn; Z = **Lys** -Glu-Glu-Asn-Gly, Arg-Gly-Asp-Ser-Pro; scu-PA143-403 = unglycosylated amino acid sequence from Glu143 to Thr403 of single-chain 54,000-Da **urokinase**-type PA) are manufactured for use as thrombolytics by expression of the gene in a microbial host. I are not bound and inactivated by specific cellular **urokinase** receptors. I (Z = Arg-Gly-Asp-Ser-Pro) show a marked affinity for blood platelets; this provides for concentration of I at its site of action. **Scu-PA** gene fragments were incorporated into a pBR322 derivative along with a synthetic multicloning site, a transcription terminator, and a tac promoter to provide plasmid pGRTac06 for expression in **Escherichia coli**. The protein product was refolded and cleaved to form active 2-chain PA.

IT 146411-89-4

RL: BIOL (Biological study)

(nucleotide sequence and expression in **Escherichia coli** of)

IT 146411-73-6 146411-74-7 146411-75-8

146411-76-9 146411-77-0

RL: PRP (Properties); BIOL (Biological study)

(nucleotide sequence of, **urokinase** analog genes containing)

L98 ANSWER 44 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1993:36558 HCAPLUS

DN 118:36558

- TI The influence of glycosylation on the catalytic and fibrinolytic properties of **pro-urokinase**
- AU Lenich, Catherine; Pannell, Ralph; Henkin, Jack; Gurewich, Victor
- CS Inst. Prevent. Cardiovasc. Dis., New England Deaconess Hosp., Boston, MA, USA
- SO Thrombosis and Haemostasis (1992), 68(5), 539-44
- CODEN: THHADQ; ISSN: 0340-6245
- DT Journal
- LA English
- AB The authors previously found that human **pro-urokinase** (**pro-UK**) expressed in **Escherichia coli** is more active in fibrinolysis than **recombinant** human **pro-UK** obtained from mammalian cell culture media. To determine whether this difference is related to the lack of glycosylation of the **E. coli** product, the authors compared the activity of **E. coli**-derived **pro-UK** [(-)**pro-UK**] with that of a glycosylated **pro-UK** [(+)**pro-UK**] and of a mutant of **pro-UK** missing the glycosylation site at Asn-302 [(-)(302)**pro-UK**]. The latter two **pro-UKs** were obtained by expression of the human gene in a mammalian cell. The nonglycosylated **pro-UKs** were activated by plasmin more efficiently ( $\approx 2$ -fold) and were more active in clot lysis (1.5-fold) than the (+)**pro-UK**. Similarly, the nonglycosylated two-chain derivs. (**UKs**) were more active against plasminogen and were more rapidly inactivated by plasma inhibitors than the (+)**UK**. These findings indicate that glycosylation at Asn-302 influences the activity of **pro-UK/UK** and could be the major factor responsible for the enhanced activity of **E. coli**-derived **pro-UK**.
- IT 82657-92-9, **Pro-urokinase**  
 RL: BIOL (Biological study)  
 (catalytic and fibrinolytic activity of, of human, glycosylation influence on)
- L98 ANSWER 45 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
- AN 1993:17442 HCAPLUS
- DN 118:17442
- TI Palindromic DNA sequence for enhanced gene expression in **Escherichia coli**
- IN Miyake, Toshio; Yamada, Masayuki; Sakaguchi, Reiko; Kubo, Miki
- PA Tosoh Corp., Japan
- SO Jpn. Kokai Tokkyo Koho, 8 pp.  
 CODEN: JKXXAF
- DT Patent
- LA Japanese
- FAN.CNT 1
- |      | PATENT NO.     | KIND | DATE     | APPLICATION NO. | DATE         |
|------|----------------|------|----------|-----------------|--------------|
| PI   | JP 04004884    | A2   | 19920109 | JP 1990-102848  | 19900420 <-- |
| PRAI | JP 1990-102848 |      | 19900420 | <--             |              |
- AB A palindromic DNA sequence useful in stabilizing mRNA transcribed in **E. coli** is provided to increase the yield of protein products. Production of human **prourokinase** derivative by **E. coli** was greatly enhanced by inserting a synthetic palindromic DNA sequence between the tac promoter and the **Shine-Dalgarno** sequence.
- L98 ANSWER 46 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN
- AN 1992:483570 HCAPLUS
- DN 117:83570
- TI Structure-function relationship of basic fibroblast growth factor: site-directed **mutagenesis** of a putative heparin-binding and

receptor-binding region

AU Presta, M.; Statuto, M.; Isacchi, A.; Caccia, P.; Pozzi, A.; Gualandris, A.; Rusnati, M.; Bergonzoni, L.; **Sarmientos, P.**

CS Sch. Med., Univ. Brescia, Brescia, 25123, Italy

SO Biochemical and Biophysical Research Communications (1992), 185(3), 1098-107

CODEN: BBRCA9; ISSN: 0006-291X

DT Journal

LA English

AB Basic residues Arg-118, Lys-119, Lys-128, and Arg-129 within a putative heparin-binding and receptor-binding region of the 155-amino acid form of basic fibroblast growth factor (bFGF) have been changed to neutral glutamine residues by site-directed **mutagenesis** of the human bFGF cDNA. The bFHF **mutant** (M6B-bFGF) was expressed in **E. coli** and purified to homogeneity. When compared to wild type bFGF, M6B-bFGF showed in cultured endothelial cells a similar receptor-binding capacity and mitogenic activity, but a reduced affinity for heparin-like low affinity binding sites, a reduced chemotactic activity, and a reduced capacity to induce the production of **urokinase**-type plasminogen activator. In vivo, M6B-bFGF lacked a significant angiogenic activity. Modifications of both the primary and the tertiary structure of bFGF appear to be responsible for the modified biol. properties of M6B-bFGF, thus confirming the possibility to dissociate at the structural level some of the biol. activities exerted by bFGF on endothelial cells.

L98 ANSWER 47 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1992:446699 HCAPLUS

DN 117:46699

TI Amidated human **prourokinase** and its manufacture by enzymic modification of the protein

IN Gozzini, Luigia; Visco, Carlo; Perego, Rita; Roncucci, Romeo; **Sarmientos, Paolo**

PA Farmitalia Carlo Erba S.r.l., Italy

SO Ger. Offen., 17 pp.

CODEN: GWXXBX

DT Patent

LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 4122688	A1	19920116	DE 1991-4122688	19910709 <--
	GB 2246133	A1	19920122	GB 1991-14846	19910710 <--
	JP 04252185	A2	19920908	JP 1991-196960	19910711 <--
PRAI	GB 1990-15369	A	19900712	<--	

AB C-terminal amidated human **prourokinase** (hPUK<sup>NH2</sup>) is prepared by expressing a gene for hPUK-Gly-Yn (n=1-4; Y=basic amino acid) in an appropriate host cell; isolating the **recombinant** protein; and treating it with carboxypeptidase B and/or an amidating enzyme such as peptidylglycine  $\alpha$ -amidating monooxygenase.

IT **82657-92-9DP, Prourokinase**, carboxy terminal-modified

RL: PREP (Preparation)

(manufacture of human, with transgenic cells, carboxy terminal-amidated **prourokinase** enzymic manufacture from)

L98 ANSWER 48 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1992:230758 HCAPLUS

DN 116:230758

TI Inhibitor-resistant analogs of **prourokinase**

IN Brandazza, Anna; Lansen, Jaqueline; Orsini, Gaetano; **Sarmientos, Paolo**

PA Farmitalia Carlo Erba S.r.l., Italy

SO Ger. Offen., 9 pp.

CODEN: GWXXBX

DT Patent

LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 4125193	A1	19920206	DE 1991-4125193	19910730 <--
	GB 2247022	A1	19920219	GB 1991-16457	19910730 <--
	JP 04252184	A2	19920908	JP 1991-190333	19910730 <--
PRAI	IT 1990-21178	A	19900802	<--	

AB **Prourokinase** analogs that are resistant to inhibition by plasminogen activator inhibitor 1 (PAI-1) are obtained by substitution of serine residues with acidic ones. Serines at positions 138, 139, or 303 are substituted with Glu or Asp. One such analog (Glu-303 **prourokinase**) was prepared by site-directed **mutagenesis** of the gene and expression of the gene in **Escherichia coli**. The analog was as active against the test substrate S2390 as the wild-type enzyme. Under conditions where the wild-type enzyme was inhibited 50% by PAI-1 this analog retained 80% of its activity.

IT **82657-92-9D, Prourokinase**, amino acid substituted analogs

RL: BIOL (Biological study)

(plasminogen activator inhibitor-resistant, manufacture in **Escherichia coli** of)

L98 ANSWER 49 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1992:230644 HCAPLUS

DN 116:230644

TI Biochemical properties of **recombinant single-chain urokinase**-type plasminogen activator **mutants** with deletion of Asn2 through Phe157 and/or substitution of Cys279 with Ala

AU Lijnen, Henri R.; Li, Xian Kui; Nelles, Luc; Hu, Mei Hao; Collen, Desire  
 CS Cent. Thromb. Vasc. Res., Univ. Leuven, Louvain, B-3000, Belg.

SO European Journal of Biochemistry (1992), 205(2), 701-9

CODEN: EJBCAI; ISSN: 0014-2956

DT Journal

LA English

AB The contribution of the NH<sub>2</sub>-terminal polypeptide chain and of the Cys148-Cys279 interchain SS bond to the enzyme activity of **urokinase**-type plasminogen activator (u-PA) was studied using site-specific **mutagenesis**. **Recombinant** single-chain u-PA (rscu-PA) variants were produced by transfecting CHO cells with cDNA encoding des(Asn2-Phe157)rscu-PA (rscu-PA with deletion of Asn2-Phe157), [Ala279]rscu-PA (rscu-PA with Cys279→Ala **mutation**) or des(Asn2-Phe157)[Ala279]rscu-PA[des(Asn2-Phe157)rscu-PA with Cys279→Ala **mutation**]. Des(Asn2-Phe157)rscu-PA, [Ala279]rscu-PA and des(Asn2-Phe157)[Ala279]rscu-PA, purified from conditioned cell culture medium, were obtained as nearly homogeneous single-chain mols. with Mr approx. 30,000, 54,000, and 30,000, and specific fibrinolytic activities on fibrin plates of 860 IU/mg, 43.0 IU/μg and 240 IU/mg, resp., compared to 69.0 IU/μg for wild-type rscu-PA obtained in the same expression system. The plasminogen-activating potential in a buffer milieu of [Ala279]rscu-PA was somewhat lower than that of rscu-PA, but that of both deletion **mutants** was virtually abolished. In a human plasma milieu in vitro, consisting of a radiolabeled human plasma clot submerged in plasma, 50% clot lysis in 2 h required 6.5 μg/mL [Ala279]rscu-PA or 3.4 μg/mL rscu-PA, whereas with both deletion **mutants** no significant clot lysis was observed with up to 16 μg/mL. Treatment of [Ala279]rscu-PA or rscu-PA with plasmin resulted in quant. conversion to two-chain mols. and was associated with an increase in specific amidolytic activity from about 600 IU/mg to 62.5 IU/μg for [Ala279]rscu-PA as compared to an increase from about

0.3 IU/ $\mu$ g to 75.0 IU/ $\mu$ g for rscu-PA. In contrast, no significant amidolytic activity could be generated by treatment of des(Asn2-Phe157)rscu-PA or des(Asn2-Phe157)[Ala279]rscu-PA with plasmin. The u-PA B-chain, isolated from plasmin-treated [Ala279]rscu-PA, had enzymic properties which were comparable to those of rtcu-PA, with respect to specific fibrinolytic activity, amidolytic activity, kinetics of plasminogen activation and clot-lysis activity in a human plasma milieu in vitro. Following bolus injection into hamsters, the plasma clearances were comparable (0.7-1.1 mL/min) for wild-type rscu-PA and for the three truncated rscu-PA mutants. These results indicate that (a) deletion of residues Asn2-Phe157 results in abolition of the enzyme activity of rscu-PA, (b) the interchain SS bond in u-PA is not required for the enzymic activity of scu-PA, (c) all the determinants required for the enzymic activity of two-chain u-PA are contained within the B-chain, and (d) the region comprising residues Asn2-Phe157 of u-PA is not required for the rapid in vivo clearance.

L98 ANSWER 50 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1992:230500 HCAPLUS

DN 116:230500

TI An enzyme-linked immunosorbent assay for urokinase-type plasminogen activator (u-PA) and mutants and chimeras containing the serine protease domain of u-PA

AU Declerck, Paul J.; Van Keer, Leen; Verstreken, Maria; Collen, Desire  
CS Cent. Thrombosis Vasc. Res., Univ. Leuven, Louvain, B-3000, Belg.

SO Thrombosis and Haemostasis (1992), 67(1), 95-100  
CODEN: THHADQ; ISSN: 0340-6245

DT Journal

LA English

AB An ELISA for quantitation of natural and recombinant plasminogen activators containing the serine protease domain (B-chain) of urokinase-type plasminogen activator (u-PA) was developed, based on two murine monoclonal antibodies, MA-4D1E8 and MA-1L3, raised against u-PA and reacting with nonoverlapping epitopes in the B-chain. MA-4D1E8 was coated on microtiter plates and bound antigen was quantitated with MA-2L3 conjugated with horseradish peroxidase. The intra-assay, inter-assay and inter-dilution coeffs. of variation of the assay were 6%, 15% and 9%, resp. Using recombinant single-chain u-PA (rscu-PA) as a standard, the u-PA-related antigen level in normal human plasma was 1.4 ng/mL. The ELISA recognized the following compds. with comparable sensitivity: intact scu-PA [amino acids (AA) 1 to 411], scu-PA-32k (AA 144 to 411), a truncated (thrombin-derived) scu-PA comprising AA 157 to 411, and chimeric t-PA/u-PA mols. including t-PA(AA1-263)/scu-PA(AA144-411), t-PA(AA1-274)/scu-PA(AA138-411) and t-PA(AA87-274)/scu-PA(AA138-411). Conversion of single-chain to two-chain forms of u-PA or inhibition of active two-chain forms with plasminogen activator inhibitor-1 or with the active site serine inhibitor phenyl-methyl-sulfonyl fluoride, did not alter the reactivity in the assay. In contrast, inactivation with  $\alpha$ 2-antiplasmin or with the active site histidine inhibitor Glu-Gly-Arg-CH<sub>2</sub>Cl resulted in a 3- to 5-fold reduction of the reactivity. When purified scu-PA-32k was added to pooled normal human plasma at final concns. ranging from 20 to 1,000 ng/mL, recoveries in the ELISA were between 84 and 110%. The assay was successfully applied for the quantitation of pharmacol. levels of scu-PA and t-PA(AA87-274)/scu-PA(AA138-411) in plasma during exptl. thrombolysis in baboons. Thus the present ELISA, which is specifically dependent on the presence of the serine protease part of u-PA, is useful for measurement of a wide variety of variants and chimeras of u-PA which are presently being developed for improved thrombolytic therapy.

L98 ANSWER 51 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1992:146703 HCAPLUS

DN 116:146703

TI **Mutations** affecting the activity of **urokinase**-type plasminogen activator

AU Davidow, Lance S.; Dumais, Dennis R.; Smyth, Adrienne P.; Greer, Jonathan; Moir, Donald T.

CS Collab. Res. Inc., Waltham, MA, 02154, USA

SO Protein Engineering (1991), 4(8), 923-8

CODEN: PRENE9; ISSN: 0269-2139

DT Journal

LA English

AB **Mutagenesis** throughout the **single-chain**

**urokinase**-type plasminogen activator (**scu-PA**)

cDNA mol., followed by expression of the **mutant** genes and secretion of the resulting **mutant** proteins from yeast, has been used to determine the amino acid residues important for activity of **scu-PA** mols. Twelve out of 13 colonies secreting variant **scu-PA** mols. with decreased ability to form a zone of fibrinolysis had **mutant** genes with a single codon alteration in the serine protease encoding domain (**B-chain**). Many of these changes are of highly conserved residues in the serine proteases and are consequently of considerable interest. A model three-dimensional structure of the protease domain of **urokinase** was used to explain the basis for the effects of these down **mutations**. The model showed that the strongest down **mutations** result from either interference of the **mutated** side chain with substrate binding at the active site or the introduction of bulky or charged groups at structurally sensitive internal positions in the mol. Attempts to find second site revertants of five down **mutants**, altered either at the plasmin activation site or near the serine at the active site, only resulted in same-site revertants, with the original or closely related amino acid restored.

L98 ANSWER 52 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1992:38583 HCAPLUS

DN 116:38583

TI A comparative study of the promotion of tissue plasminogen activator and **pro-urokinase**-induced plasminogen activation by fragments D and E-2 of fibrin

AU Liu, Jian Ning; Gurewich, Victor

CS Inst. Prevent. Cardiovasc. Dis., New England Deaconess Hosp., Boston, MA, 02215, USA

SO Journal of Clinical Investigation (1991), 88(6), 2012-17

CODEN: JCINAO; ISSN: 0021-9738

DT Journal

LA English

AB Plasmin generation by equimolar concns. of tissue plasminogen activator (**t-PA**), **pro-urokinase** (**pro-UK**), and **urokinase** (**UK**), and a 2-fold higher concentration of a plasmin-resistant **mutant rpro-UK** (Ala-158-**pro-UK**) was measured on a microtiter plate reader. The promoting effects on this reaction of equimolar concns. of fibrinogen, soluble fibrin (Desafib), CNBr fragment FCB-2 (an analog of fragment D), or purified fragment E-2 were compared. Plasmin generation by **t-PA** was moderately promoted by fibrinogen, substantially promoted by Desafib and FCB-2, but not at all promoted by fragment E-2. By contrast, plasmin generation by **pro-UK** or by Ala-158-**pro-UK** was not promoted either by fibrinogen, Desafib, or FCB-2, but was significantly promoted by any of the fibrin(ogen) preps. Treatment of fragment E-2 by carboxypeptidase-B (CPB), eliminated its promotion of **pro-UK** and Ala-158-**pro-**

UK-induced plasmin generation. Pretreatment of FCB-2 with plasmin slightly potentiated its promotion of t-PA activity. This effect of plasmin pretreatment of FCB-2 was reversed by CPB treatment. Plasmin pretreatment of FCB-2 did not induce any promotion of activity in **pro-UK** or Ala-158-**pro-UK**. The findings show that the intrinsic activity of **pro-UK** and the activity of t-PA are promoted by different regions of the fibrin(ogen) mol. The latter is stimulated primarily by a determinant in the fragment D region, which is available in intact fibrin. By contrast, plasminogen activation by the intrinsic activity of **pro-UK** was stimulated exclusively by fragment E-2, which is unavailable in intact fibrin. The findings are believed relevant to fibrinolysis and support the concept that t-PA and **pro-UK** are complementary, sequential, and synergistic in their actions.

IT 82657-92-9, Pro-urokinase

RL: BIOL (Biological study)

(plasminogen activation induction by, fibrinogen degradation products D and E2 effect on)

L98 ANSWER 53 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1991:672471 HCAPLUS

DN 115:272471

TI Use of the **urokinase**-type plasminogen activator gene as a general tool to monitor expression in transgenic animals: study of the tissue-specificity of the murine whey acidic protein (WAP) expression signals

AU Brandazza, Anna; Lee, Eric; Ferrera, Monica; Tillman, Ulrich; **Sarmientos, Paolo**; Westphal, Heiner

CS Dep. Biotechnol., Farmitalia Carlo Erba, Milan, 20146, Italy

SO Journal of Biotechnology (1991), 20(2), 201-12

CODEN: JBITD4; ISSN: 0168-1656

DT Journal

LA English

AB **Urokinase**-type plasminogen activator (uPA) is a proteolytic enzyme able to convert the zymogen plasminogen into the strong protease plasmin. The availability of very sensitive tests to measure the enzymic activity of a plasminogen activator renders the corresponding gene an ideal candidate for the detection of promoter activity. In this paper the authors describe the utilization of the human uPA gene as detector of tissue-specificity of the murine whey acidic protein (WAP) expression signals in transgenic mice. The WAP promoter has been previously investigated for the production of foreign proteins in the milk of transgenic animals. In the genetic constructions prepared here, the human uPA cDNA was linked to the promoter region as well as to 3'-end distal sequences of the WAP gene. Five transgenic lines were obtained in which, however, expression levels of human uPA in the milk were still quite low. Surprisingly, 4 of these 5 pos. transgenic mice show a consistent activity of the WAP promoter in brain exts. compared to other tissues.

L98 ANSWER 54 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1991:649567 HCAPLUS

DN 115:249567

TI Modification of **Shine/Dalgarno** sequence and its effect on gene expression

IN Kubo, Miki; Higo, Hirohito

PA Tosoh Corp., Japan

SO Jpn. Kokai Tokkyo Koho, 8 pp.

CODEN: JKXXAF

DT Patent

LA Japanese

FAN.CNT 1

PATENT NO.

KIND

DATE

APPLICATION NO.

DATE

PI JP 03094686 A2 19910419 JP 1989-233281 19890908 <--  
 PRAI JP 1989-233281 19890908 <--

AB The **Shine/Dalgarno** (SD) sequence of a gene or the DNA sequence is modified by substitution to ease/hinder the formation of secondary structure, i.e. to change the free energy required for pairing between 2 bases. By such modification the gene expression, and therefore the protein production, can be regulated in a **recombinant** microbial host. The SD sequence and its surrounding regions of **prourokinase** gene (on plasmid pMUTBS), that formed a secondary structure with a free energy of -10.2 kcal/mol, was modified with 2 synthetic oligonucleotides to increase the free energy to 0 (hindered). Plasmid pMUTRE carrying the modified gene was prepared and used for transformation of **Escherichia coli**. **Prourokinase** produced by the transformants was 4-fold higher than the control.

IT 82657-92-9, **Prourokinase**

RL: PRP (Properties)

(gene for, expression in **Escherichia coli** of, effect of internal secondary **Shine Dalgarno** structure on)

L98 ANSWER 55 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1991:221530 HCAPLUS

DN 114:221530

TI A six-amino acid deletion in basic fibroblast growth factor dissociates its mitogenic activity from its plasminogen activator-inducing capacity

AU Isacchi, Antonella; Statuto, Massimo; Chiesa, Roberta; Bergonzoni, Laura; Rusnati, Marco; **Sarmientos, Paolo**; Ragnotti, Giovanni; Presta, Marco

CS Sch. Med., Univ. Brescia, Brescia, 25124, Italy

SO Proceedings of the National Academy of Sciences of the United States of America (1991), 88(7), 2628-32

CODEN: PNASA6; ISSN: 0027-8424

DT Journal

LA English

AB A **recombinant** deletion mutant of the 155-amino acid form of human basic fibroblast growth factor (bFGF), lacking amino acid residues 27-32 (Lys-Asp-Pro-Lys-Arg-Leu), was expressed in **Escherichia coli** and purified to homogeneity by heparin-Sepharose affinity chromatog. When maintained in the presence of an equimolar concentration of soluble heparin, the bFGF mutant (M1-bFGF) is as potent as bFGF in stimulating cell proliferation in normal and transformed fetal bovine aortic endothelial cells, in adult bovine aortic endothelial cells, and in NIH 3T3 fibroblasts. However, under the same exptl. conditions, M1-bFGF is at least 100-fold less efficient than bFGF in stimulating plasminogen activator (PA) production in endothelial cells, as assayed by chromogenic PA assay, SDS/PAGE zymog., and Northern blot anal. of **urokinase**-type PA mRNA. In the presence of heparin, M1-bFGF binds to bFGF plasma membrane receptors present on endothelial cells in a manner indistinguishable from that of bFGF. It also induces the same tyrosine phosphorylation pattern when added to NIH 3T3 cells. Thus, the PA-inducing activity of bFGF may depend upon a functional domain that differs from those involved in the mitogenic activity of the growth factor, and the binding of bFGF to its plasma membrane receptor may not be sufficient to induce **urokinase**-type PA production in endothelial cells.

L98 ANSWER 56 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1991:156602 HCAPLUS

DN 114:156602

TI Efficient renaturation and fibrinolytic properties of **prourokinase** and a deletion mutant expressed in **Escherichia coli** as inclusion bodies

- AU Orsini, Gaetano; Brandazza, Anna; Sarmientos, Paolo; Molinari,  
Antonio; Lansen, Jacqueline; Cauet, Gilles  
CS Dep. Biotechnol., Farmitalia C, Erba, Milan, I-20146, Italy  
SO European Journal of Biochemistry (1991), 195(3), 691-7  
CODEN: EJBCAI; ISSN: 0014-2956  
DT Journal  
LA English  
AB **Prourokinase** is a plasminogen activator of 411 amino acids which displays clot-lysis activity through a fibrin-dependent mechanism, and which seems to be a promising agent for the treatment of acute myocardial infarction. The preparation of **recombinant prourokinase** in bacteria has been hampered by its insoly. and by difficulty in refolding the polypeptide chain. In this paper the authors describe the renaturation process of 2 **recombinant** proteins expressed in **E. coli** as inclusion bodies: **prourokinase** and a deletion derivative ( $\Delta$ 125- **prourokinase**) in which 125 amino acids of the N-terminal region have been removed. Deletion of this sequence brings higher refolding yields and faster kinetics (first-order rate constant of renaturation of 0.57/h for  $\Delta$ 125- **prourokinase** and 0.25/h for **prourokinase**). The process involves sequential steps of denaturation, reduction and controlled refolding of the polypeptide chain. When applied to pure, non-glycosylated and active **prourokinase**, it gives a refolding yield of about 80%, demonstrating the efficiency of the renaturation procedure. Lower yields (15 and 30%, resp., for **prourokinase** and  $\Delta$ 125- **prourokinase**) were obtained when the same refolding protocol was applied to inclusion bodies from bacteria. After purification to homogeneity (as shown by HPLC and SDS/PAGE) specific activities were 160000 and 250000 IU/mg protein, resp., for **prourokinase** and  $\Delta$ 125- **prourokinase**. As with **prourokinase**, the deletion **mutant**  $\Delta$ 125- **prourokinase** displays a zymogenic nature, being activated by plasmin to the active two-chain form; however, this **mutant** is approx. 4-fold more resistant than **prourokinase** to plasmin activation, and consequently shows a different fibrinolytic profile.
- IT 82657-92-9, **Prourokinase**  
RL: BIOL (Biological study)  
(efficient renaturation and fibrinolytic property of a deletion **mutant** expressed in **Escherichia coli** as inclusion bodies and, structure in relation to)
- L98 ANSWER 57 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 1991:137351 HCAPLUS  
DN 114:137351  
TI Optimizing the promoter and ribosome binding sequence for expression of human **single chain urokinase**-like plasminogen activator in **Escherichia coli** and stabilization of the product by avoiding heat shock response  
AU Surek, Baerbel; Wilhelm, Martin; Hillen, Wolfgang  
CS Inst. Mikrobiol. Biochem., Friedrich-Alexander-Univ., Erlangen, D-8520, Germany  
SO Applied Microbiology and Biotechnology (1991), 34(4), 488-94  
CODEN: AMBIDG; ISSN: 0175-7598  
DT Journal  
LA English  
AB The expression of **recombinant single-chain urokinase**-like plasminogen activator (rscuPA) in **Escherichia coli** was optimized by fusing the **puk** gene to different promoters and ribosome binding sequences.. Comparison of the tac, trp and  $\lambda$ PL promoters showed that expression was maximal under tac control. Variation in the ribosome binding sequence and its distance to the AUG start codon yielded a further slight improvement of expression. The largest increase in rscuPA expression was achieved by

variations in the host strain and growth conditions. In *E. coli* DG75 grown at 37° maximal expression was achieved 30 min after induction and decreased gradually until 240 min after induction. Growth at 30° yielded maximal expression 60 min after induction and resulted in reduced activity at longer times. Western blot anal. of the products showed that degradation of rscuPA was much larger at 37° than at 30°. Using *E. coli* CAG630 carrying the *htpR* mutation, which avoids heat shock response, for expression of rscuPA eliminated the instability of the product at both temps. Expression in this strain was even more efficient than in *E. coli* JM101 carrying the *lon* mutation. It is concluded that induction of the general heat-shock response in *E. coli* must be avoided to obtain stabilization of rscuPA. This drastically improves the overall yield of rscuPA from recombinant *E. coli* strains.

L98 ANSWER 58 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1991:117685 HCAPLUS

DN 114:117685

TI Stabilization of freeze-dried **prourokinase**

IN Morimoto, Kazuo; Narita, Shusaku; Nishikawa, Masaru; Takechi, Kazuo

PA Green Cross Corp., Japan

SO Eur. Pat. Appl., 6 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 391400	A2	19901010	EP 1990-106492	19900405 <--
	EP 391400	A3	19901114		
	R: BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE				
	JP 02268681	A2	19901102	JP 1989-86932	19890407 <--
	CA 2014009	AA	19901007	CA 1990-2014009	19900406 <--
PRAI	JP 1989-86932	A	19890407	<--	

AB **Prourokinase** for use in pharmaceuticals is stabilized as a dry powder by incorporating a polar amino acid (acidic or basic) or their salts into the **prourokinase** solution before freeze drying. The amino acid is added at 5-30 mg per 10,000-250,000 IU **prourokinase**. Samples of **prourokinase** 25,000 IU in phosphate buffer were freeze-dried in the presence of 6.4 mg/mL of aspartate, glutamate, Na glutamate, arginine, **lysine**, or **histidine**. Recovery of activity immediately after lyophilization was 96% in all cases (94% in a control with no addns.). After 3 mo at 50° the test samples retained 56-63% of activity whereas the control sample (no addns.) had retained only 2% of its activity. This stabilization was also shown to be a function of the concentration of the stabilizer.

IT 82657-92-9, **Prourokinase**

RL: BIOL (Biological study)

(freeze-dried, stabilization of, polar amino acids for)

IT 56-87-1, **Lysine**, biological studies 71-00-1,

**Histidine**, biological studies

RL: BIOL (Biological study)

(**prourokinase** stabilized by, after freeze-drying)

L98 ANSWER 59 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1991:69055 HCAPLUS

DN 114:69055

TI Pharmaceutical composition comprising a plasminogen activator and hirudin

IN Heim, Jutta; Agnelli, Giancarlo; Czendlik, Czeslaw

PA Ciba-Geigy A.-G., Switz.; UCP Gen-Pharma A.-G.

SO Eur. Pat. Appl., 33 pp.

CODEN: EPXXDW

DT Patent  
LA English  
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 365468	A1	19900425	EP 1989-810676	19890912 <--
	EP 365468	B1	19940216		
	R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
	AT 101524	E	19940315	AT 1989-810676	19890912 <--
	AU 8941352	A1	19900329	AU 1989-41352	19890913 <--
	AU 628854	B2	19920924		
	US 5126134	A	19920630	US 1989-408836	19890918 <--
	CA 1336493	A1	19950801	CA 1989-611903	19890919 <--
	DK 8904639	A	19900322	DK 1989-4639	19890920 <--
	JP 02121934	A2	19900509	JP 1989-242356	19890920 <--
	ZA 8907173	A	19900926	ZA 1989-7173	19890920 <--
	IL 91706	A1	19970713	IL 1989-91706	19890920 <--
	KR 149001	B1	19981015	KR 1989-13492	19890920 <--
PRAI	GB 1988-22147	A	19880921	<--	
	EP 1989-810676	A	19890912	<--	

AB Pharmaceutical compns. containing a plasminogen activator and a hirudin can be used for prophylaxis and therapy of thrombosis or diseases caused by thrombosis. The dissoln. of thrombi is accelerated significantly and the risk of reocclusion is considerably reduced when using this combination rather than a plasminogen activator alone. Lysis of thrombi were observed with a rabbit jugular vein thrombosis model; i.v. administration of tissue plasminogen activator (tPA) alone, of tPA and heparin, and of tPA and hirudin produced 37-44, 34, and 52% clot lysis, resp. Addnl., the presence of hirudin decreased thrombin accretion by approx. 50% relative to tPA alone or to tPA and heparin. Expression vectors for manufacture of hirudin mutants in *Escherichia coli* and yeast were prepared

IT 121449-21-6 121449-35-2

RL: BIOL (Biological study)

(as thrombolytic, improved rate of clot lysis and inhibition of thrombus accretion with)

IT 131790-70-0 131790-74-4 131790-75-5

131877-61-7

RL: BIOL (Biological study)

(as thrombolytic, increased rate of clot lysis and decreased clot accretion with)

L98 ANSWER 60 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1991:18956 HCAPLUS

DN 114:18956

TI Manufacture of prourokinase with *Escherichia coli*

IN Brandazza, Anna; Sarmientos, Paolo; Orsini, Gaetano

PA Farmitalia Carlo Erba S.r.l., Italy

SO PCT Int. Appl., 38 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9004023	A1	19900419	WO 1989-EP1168	19891006 <--
	W: AT, DK, FI, HU, JP, KR, NO, SU, US				
	RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
	EP 365894	A1	19900502	EP 1989-118586	19891006 <--
	R: ES, GR				
	EP 407490	A1	19910116	EP 1989-911367	19891006 <--
	R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE				

HU 55443	A2	19910528	HU 1989-6054	19891006 <--
HU 209149	B	19940328		
JP 03502526	T2	19910613	JP 1989-510599	19891006 <--
CA 2000408	AA	19900411	CA 1989-2000408	19891010 <--
CN 1042181	A	19900516	CN 1989-108587	19891010 <--
AU 8943823	A1	19910502	AU 1989-43823	19891026 <--
AU 624869	B2	19920625		
NO 9002564	A	19900809	NO 1990-2564	19900608 <--
DK 9001410	A	19900813	DK 1990-1410	19900608 <--
US 5866358	A	19990202	US 1990-536556	19900711 <--
PRAI GB 1988-23833	A	19881011	<--	
WO 1989-EP1168	W	19891006	<--	

AB Human **prourokinase** is manufactured with **recombinant E. coli**. The **prourokinase** cDNA is expressed in **E. coli B** under the control of the P<sub>trp</sub> promoter and the **Shine-Dalgarno** sequence MS-2.

L98 ANSWER 61 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1990:545081 HCAPLUS

DN 113:145081

TI Characterization of thrombin- and plasmin-resistant **mutants** of **recombinant human single chain urokinase-type plasminogen activator**

AU Eguchi, Yutaka; Sakata, Yoichi; Matsuda, Michio; Osada, Hiroshi; Numao, Naganori; Ohmori, Muneki; Kondo, Kiyoshi

CS Inst. Hematol., Jichi Med. Sch., Tochigi, 329-04, Japan

SO Journal of Biochemistry (Tokyo, Japan) (1990), 108(1), 72-9

CODEN: JOBIAO; ISSN: 0021-924X

DT Journal

LA English

AB **Recombinant human single-chain**

**urokinase-type plasminogen activator (Scu-PA)**

(SM0:wild type) and its variants resistant to plasmin and/or thrombin

(SM1:Lys135 → Gln; SM3:Phe157 → Asp; and SM4:Lys135 →

Gln and Phe157 → Asp) have been constructed by site-directed

**mutagenesis** with the aim of producing more efficient thrombolytic

agents. The **recombinant** variant **scu-PAs**

expressed in **Escherichia coli** were characterized.

They appeared to have structural integrity because their heat stabilities, immunol. reactivities, and CD spectra were essentially identical to each other and to those of native **scu-PA** (nscu-PA). In the

presence of thrombin, SM3 and SM4 caused efficient clot lysis in all of the assays used, compared with SM0, SM1, and nscu-PA. In the absence of thrombin, when measured by a fibrin plate method in a purified system, SM3 and SM4 had lower sp. activities than SM0, SM1, and nscu-PA, since their catalytic consts. for conversion to the 2-chain form by plasmin were lower. SM4 lysed clots as efficiently as SM0 in plasma by retaining the single-chain form, whereas SM0 was partly converted to the 2-chain form.

L98 ANSWER 62 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1990:491463 HCAPLUS

DN 113:91463

TI Low molecular weight derivatives of **prourokinase** as thrombolytics

IN Brandazza, Anna; Lansen, Jacqueline; Mazue, Guy; **Sarmientos, Paolo**

PA Farmitalia Carlo Erba S.r.l., Italy

SO Eur. Pat. Appl., 16 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

PATENT NO.

KIND

DATE

APPLICATION NO.

DATE

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PI EP 338409 A1 19891025 EP 1989-106549 19890413 <--  
 EP 338409 B1 19950201  
 R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, NL, SE  
 WO 8910402 A1 19891102 WO 1989-EP394 19890413 <--  
 W: AU, DK, FI, HU, JP, KR, NO, SU, US  
 AU 8934149 A1 19891124 AU 1989-34149 19890413 <--  
 AU 617459 B2 19911128  
 HU 52558 A2 19900728 HU 1989-2527 19890413 <--  
 JP 03500125 T2 19910117 JP 1989-504110 19890413 <--  
 CN 1037360 A 19891122 CN 1989-102351 19890418 <--  
 ZA 8902829 A 19900131 ZA 1989-2829 19890418 <--  
 IL 90011 A1 19941021 IL 1989-90011 19890418 <--  
 NO 8905078 A 19900216 NO 1989-5078 19891215 <--  
 DK 8906416 A 19891218 DK 1989-6416 19891218 <--

PRAI GB 1988-9093 A 19880418 <--  
 WO 1989-EP394 A 19890413 <--

AB Low mol.-weight derivs. of human **prourokinase** are provided which comprise at least the sequence from amino acid 163 to amino acid 411 of mature **prourokinase** in single chain configuration. In preferred cases, the sequences making up the kringle domain, the receptor binding domain or cell binding domain are missing. The derivs. show thrombolytic activity. Modified genes encoding for these derivs. were constructed by site-specific **mutagenesis**. Using traditional expression plasmids, the **mutated** genes were expressed in **recombinant Escherichia coli** strains. The amidolytic behavior of the new derivs.  $\Delta 125$ ,  $\Delta 140$  and  $\Delta 150$   $\Delta$  = deleted amino acid sequences indicated that any mol. which keeps at least 261 amino acids of **prourokinase** in a single chain configuration could be fully amidolytically active upon plasmin activation by conversion in the two-chain configuration. Heparin, in concns. which can be reached in plasma during therapeutic anticoagulation (0.01-1 IU/mL), concomitantly to thrombolytic therapy, stimulated the conversion of  $\Delta 125$  in its two-chain configuration, by plasmin. This effect was also obtained using wild-type **prourokinase**, but to a much lesser extent. Almost complete lysis of  $^{125}\text{I}$ -labeled human fibrin clots were obtained with the derivs., without fibrinogenolysis, which was not the case for two-chain **urokinase**. This effect was maintained after  $\geq 24$  h incubation of the derivs. in plasma prior to the addition of the  $^{125}\text{I}$ -labeled human fibrin clot. On the contrary, two-chain **urokinase** clot lysis activity disappeared completely after long term pre-incubation in human plasma. Long half-life in the blood was observed after single i.v. injection of  $\Delta 125$ .

IT 82657-92-9DP, **Prourokinase**, low-mol. weight derivs.  
 RL: SPN (Synthetic preparation); PREP (Preparation)  
 (preparation of, as thrombolytic agents)

L98 ANSWER 63 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1990:401558 HCAPLUS

DN 113:1558

TI **Recombinant human single-chain urokinase-type plasminogen activator mutant** produced by site-specific **mutagenesis** of **lysine 158** to **histidine 158**

IN Collen, Desire Jose; Nelles, Lucien Georges Raymond; Lijnen, Henri Roger; De Cock, Frans; Van Hoef, Berthe; Stassen, Jean Marie

PA Leuven Research and Development V.Z.W., Belg.

SO Eur. Pat. Appl., 15 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI EP 336508 A1 19891011 EP 1989-200825 19890330 <--  
 R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE  
 PRAI US 1988-178205 A 19880406 <--  
 AB Single chain human [His158] **urokinase** has kinetic and inactivation properties and in vivo behavior in animal test systems that make it suitable for use in the treatment of thromboembolism. A cDNA encoding this protein was prepared by standard site-directed **mutagenesis** methods and the protein manufactured by expression of the gene in CHO cells. The purified protein was converted to the two-chain form by plasmin but not by thrombin. Activation of plasminogen by this protein was as effective as that of unmodified **urokinase**. Tests of stimulation of thrombolysis in vivo (rabbit jugular vein model) showed a linear dose-response curve in the range 0.5-2.0 mg **urokinase**/kg.

L98 ANSWER 64 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1990:133816 HCAPLUS

DN 112:133816

TI **Recombinant** manufacture of thrombin-resistant **prourokinase** derivatives with amino acid substitutions at position 156

IN Koerwer, Wolfgang; Kurfuerst, Manfred; Baldinger, Verena; Doerper, Thomas

PA BASF A.-G., Fed. Rep. Ger.

SO Eur. Pat. Appl., 20 pp.

CODEN: EPXXDW

DT Patent

LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 312942	A2	19890426	EP 1988-117188	19881015 <--
	EP 312942	A3	19900502		
	R: AT, BE, CH, DE, ES, FR, GB, IT, LI, NL				
	DE 3735916	A1	19890503	DE 1987-3735916	19871023 <--
	DK 8805875	A	19890614	DK 1988-5875	19881021 <--
	JP 01160482	A2	19890623	JP 1988-266255	19881024 <--
PRAI	DE 1987-3735916	A	19871023	<--	

AB **Prourokinases** with amino acid substitutions at position 156 which are resistant to thrombin inhibition are prepared by in vitro **mutagenesis** of a cDNA and manufactured by expression in **Escherichia coli**. These derivs. have properties better suited for use as pharmaceuticals. **Prourokinases** with arginine-156 replaced by glutamic acid or leucine were prepared by oligonucleotide-directed site-specific **mutagenesis** and manufactured by expression in **E. coli**. The inhibition of amidolytic activity by thrombin was compared. Although the normal **prourokinase** is reduced to 36% of control activity by incubation with 10 units of thrombin at 37° for 30 min, the substituted derivs. retained 100% activity after 1 h incubation under the same conditions.

IT 125959-05-9 125959-06-0 125959-07-1

RL: PRP (Properties); BIOL (Biological study)  
 (nucleotide sequence and expression in **Escherichia coli** of)

IT 82657-92-9D, **Prourokinase**, derivs.

RL: PRP (Properties)  
 (thrombin-resistant of, cloning and expression in **Escherichia coli** of cDNA for)

L98 ANSWER 65 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1990:133815 HCAPLUS

DN 112:133815

TI Polypeptides with a **prourokinase** activity, their production and use

IN Koerwer, Wolfgang; Kurfuerst, Manfred; Baldinger, Verena; Doerper, Thomas;  
Schwarz, Margarete  
PA BASF A.-G., Fed. Rep. Ger.  
SO Eur. Pat. Appl., 21 pp.  
CODEN: EPXXDW

DT Patent  
LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 312941	A2	19890426	EP 1988-117186	19881015 <--
	EP 312941	A3	19900516		
	R: AT, BE, CH, DE, ES, FR, GB, IT, LI, NL				
	DE 3735917	A1	19890503	DE 1987-3735917	19871023 <--
	DK 8805876	A	19890614	DK 1988-5876	19881021 <--
	JP 01160481	A2	19890623	JP 1988-266254	19881024 <--
PRAI	DE 1987-3735917	A	19871023	<--	

AB **Prourokinases** with amino acid substitutions at position 156 and N-terminal extensions resistant to thrombin inhibition and with prolonged half-lives in vivo are prepared by in vitro **mutagenesis** of a cDNA and manufactured by expression in **Escherichia coli**. These derivs. have properties better suited for use as pharmaceuticals. Polypeptides with an addnl. N-terminal Met and Arg156 (I) or Lys156 (II) were injected into male Sprague-Dawley rats at 1 mg/kg. The half-life of the naturally-occurring form of the protein was 3 min whereas for I it was 10 min and  $\geq 10$  min for II.

IT 125959-08-2 125959-09-3

RL: PRP (Properties); BIOL (Biological study)  
(nucleotide sequence and expression in **Escherichia coli** of)

IT 82657-92-9D, **Prourokinase**, derivs.

RL: PRP (Properties)  
(thrombin resistant, long serum half-life, cDNA for, cloning in **Escherichia coli** of)

L98 ANSWER 66 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1990:113619 HCAPLUS

DN 112:113619

TI Human **prourokinase** variants and their recombinant manufacture with **Escherichia coli**

IN Tagawa, Michito; Kobayashi, Yoichi; Yamada, Masayuki; Wada, Masakatsu; Mukohara, Yukio; Fushimi, Takaomi; Hachiman, Hideo; Omori, Muneki; Yokoyama, Midori; Et, Al.

PA Sagami Chemical Research Center, Japan; Central Glass Co., Ltd.; Hodogaya Chemical Co., Ltd.

SO Jpn. Kokai Tokkyo Koho, 15 pp.

CODEN: JKXXAF

DT Patent

LA Japanese

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 01187087	A2	19890726	JP 1988-9369	19880119 <--
PRAI	JP 1988-9369		19880119	<--	

AB Human **prourokinase** mutants with amino acid substitutions at Lys-135 or Phe-157, or at both, are manufactured by expression of the mutated genes in **Escherichia coli** from plasmids from specified regulatory sequences such as the trp promoter/operator, or tac or lac or lacUV5 promoter/operator in combination with an upstream lac repressor such as lacIq. Expression plasmids pUKO2pm4, pRQPSDpm4, and pRQCSdp4 encoding [Gln-135, Asp-157] **prourokinase** and expression plasmid pUKO1pm1 encoding [Gln-135] **prourokinase** were constructed and used for gene expression with

yields 2-3.5-fold higher than the prior art.

IT 82657-92-9

RL: PRP (Properties)

(amino acid substituted, gene for, expression in *Escherichia coli* of)

L98 ANSWER 67 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1990:94567 HCAPLUS

DN 112:94567

TI A recombinant pro-urokinase derived

mutant missing the growth factor-like domain does not bind to its receptor

AU Robbiati, F.; Nolli, M. L.; Soffientini, A.; Sarubbi, E.; Stoppelli, M. P.; Cassani, G.; Parenti, F.; Blasi, F.

CS Lepetit Res. Cent., Merrel Dow Res. Inst., Gerenzano, 21040, Italy

SO Fibrinolysis (1990), 4(1), 53-60

CODEN: FBRIE7; ISSN: 0268-9499

DT Journal

LA English

AB Prourokinase (pro-u-PA) is the

single chain precursor of the urokinase-type plasminogen

activator u-PA. Both u-PA and pro-u-PA bind

to an extracellular receptor present on the membrane of several cell types of both malignant and normal origin, including endothelial cells.

Competition expts. with u-PA fragments and synthetic peptides have suggested that the growth factor-like domain (GFD) of u-PA is involved in

receptor binding. To prove the direct involvement of the GFD in receptor binding, a mutant u-PA gene missing the GFD was constructed and

expressed. Mutant 9Y45 has u-PA introns C and D fused together,

thus deleting exon IV that codes for the GFD (amino acids 9-45). The

product of the mutant gene is a single chain, pro-

u-PA derived protein with an apparent Mr .apprx.43 kDa

that, upon conversion to the 2 chain form, acquires full enzymic activity, suggesting that the mol. has preserved most of its original structure.

Mutant 9Y45 is recognized by an anti-human u-PA serum, by a

monoclonal antibody directed against the kringle domain but not by a

monoclonal raised against the GFD. N-terminal and C-terminal amino acid

sequencing of the purified protein confirms that: (a) the GFD is absent; (

b) the difference in MW is not due to a truncated protein; (c) the

missing amino acids 9-45 are substituted by a novel tyrosine joining the last amino acid in exon III to the first amino acid of exon V.

Mutant 9Y45 does not bind to the u-PA receptor as shown by its

inability to compete with 125I-labeled DFP-treated u-PA for binding to the u-PA receptor of human U937 cells, even at a concentration 1000-fold higher

than

that of control pro-u-PA. Thus, the most

important receptor binding determinants of pro-u-

PA (and u-PA) reside in the GFD.

IT 82657-92-9, Prourokinase

RL: PRP (Properties)

(growth factor-like domain of, urokinase-type plasminogen activator-receptor binding requirement for)

L98 ANSWER 68 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1989:513738 HCAPLUS

DN 111:113738

TI *Escherichia coli* with lon gene suppression and its use

for efficient manufacture of recombinant proteins

IN Tagawa, Naoto; Yamada, Masayuki; Kakiya, Hitoshi; Numao, Osanori; Yura, Takashi

PA Sagami Chemical Research Center, Japan; Central Glass Co., Ltd.; Hodogaya Chemical Co., Ltd.; Nippon Soda Co., Ltd.; Nissan Chemical Industries, Ltd.

SO Jpn. Kokai Tokkyo Koho, 6 pp.

CODEN: JKXXAF

DT Patent

LA Japanese

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 63129981	A2	19880602	JP 1986-274105	19861119 <--
	JP 07077555	B4	19950823		
PRAI	JP 1986-274105		19861119	<--	

AB **Escherichia coli** deficient in tRNA suppressor activity (sup-) and lon gene expression (due to **mutation** of the htpR gene) in the temperature range optimal for cell growth or gene expression for heterologous proteins is used for manufacturing the proteins. **E. coli** strain KY1436 transformed with plasmid pHA20 encoding plasminogen activator was cultivated in L medium (5 mL) at 30° with agitation until the cell concentration reached OD600 0.4, followed by shifting

to

37° and cultivating an addnl. 6 h. I was recovered from the cells and identified by SDS-PAGE.

IT **82657-92-9P, Prourokinase**

RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)

(manufacture of, with **Escherichia coli** with inactivated lon gene and tRNA suppressor)

L98 ANSWER 69 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1989:434671 HCAPLUS

DN 111:34671

TI Single-stranded hybrid plasminogen activators, their **recombinant** manufacture, and pharmaceutical compositions containing them

PA Ciba-Geigy A.-G., Switz.

SO Jpn. Kokai Tokkyo Koho, 72 pp.

CODEN: JKXXAF

DT Patent

LA Japanese

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 63160581	A2	19880704	JP 1987-306016	19871204 <--
	JP 2641875	B2	19970820		
	FI 8705324	A	19880606	FI 1987-5324	19871202 <--
	FI 100106	B1	19970930		
	EP 277313	A1	19880810	EP 1987-117892	19871203 <--
	EP 277313	B1	19970122		
	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	IL 84700	A1	19921115	IL 1987-84700	19871203 <--
	AT 148167	E	19970215	AT 1987-117892	19871203 <--
	ES 2095825	T3	19970301	ES 1987-117892	19871203 <--
	DK 8706381	A	19880606	DK 1987-6381	19871204 <--
	DK 175483	B1	20041108		
	NO 8705069	A	19880606	NO 1987-5069	19871204 <--
	NO 177603	B	19950710		
	NO 177603	C	19951018		
	AU 8782091	A1	19880616	AU 1987-82091	19871204 <--
	AU 621281	B2	19920312		
	JP 09117292	A2	19970506	JP 1996-260999	19871204 <--
	US 5242819	A	19930907	US 1991-808936	19911213 <--
	US 5580559	A	19961203	US 1994-311848	19940923 <--
PRAI	GB 1986-29153	A	19861205	<--	
	GB 1987-1160	A	19870120	<--	
	GB 1987-9656	A	19870423	<--	
	GB 1987-15890	A	19870706	<--	

US 1987-125039 B2 19871123 <--  
 JP 1987-306016 A3 19871204 <--  
 US 1989-361015 B1 19890602 <--  
 US 1991-808936 A3 19911213 <--  
 US 1993-49469 B1 19930419 <--

AB **Recombinant** single-stranded human hybrid plasminogen activators (PA) comprised of part or all of **urokinase**-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA), or muteins thereof, are prepared. The novel hybrid PAs have improved affinity for fibrin and stability in vivo. Plasmid pJDB207/PH05-I-MOUK2TPAB comprising the PH05 gene promoter, the invertase gene signal sequence, and a **chimeric** gene encoding the kringle 2 domain of uPA fused to the B-chain of tPA [i.e., uPA(1-44)-tPA(176-527)] was constructed. This hybrid PA, i.e. UK2TPAB, was recovered from the cell homogenate of transformed *Saccharomyces cerevisiae* and purified. with monoclonal antibodies and chromatog. The thrombolytic activity of UK2TPAB was similar to t-PA, but its half-life in peripheral blood of rabbits was 10 times greater (20 vs. 2 min). Parenteral preparation containing UK2TPAB was prepared

IT 121449-18-1 121449-19-2 121449-20-5  
 121449-21-6 121449-22-7 121449-23-8  
 121449-24-9 121449-25-0 121449-26-1  
 121449-27-2 121449-28-3 121449-29-4  
 121449-30-7 121449-33-0 121449-34-1  
 121449-35-2

RL: PRP (Properties)

(amino acid sequence of and cloning and expression in eukaryotes of cDNA for)

L98 ANSWER 70 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1989:2184 HCAPLUS

DN 110:2184

TI **Recombinant urokinase**-tissue plasminogen activator fusion proteins

IN Bollen, Alex Joseph; Gheysen, Dirk; Jacobs, Paul; Pierard, Laurent; Collen, Desire J.

PA Smith Kline-RIT S. A., Belg.; Leuven Research and Development VZW

SO PCT Int. Appl., 51 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 8804690	A1	19880630	WO 1987-BE18	19871211 <--
	W: AU, DK, JP				
	ZA 8709286	A	19881026	ZA 1987-9286	19871210 <--
	AU 8811035	A1	19880715	AU 1988-11035	19871211 <--
	EP 275856	A1	19880727	EP 1987-870176	19871211 <--
	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	JP 02501106	T2	19900419	JP 1987-506364	19871211 <--
	DK 8804590	A	19880816	DK 1988-4590	19880816 <--
PRAI	US 1986-942127	A	19861216	<--	
	WO 1987-BE18	A	19871211	<--	

AB Plasmids encoding fusion proteins comprising tissue-type plasminogen activator chain A-derived peptide and **urokinase** chain B -derived peptide are constructed and expressed in mammalian cells. Plasmid pULB9151, containing DNA encoding a 262 amino acid N-terminal peptide from tissue plasminogen activator fused to DNA encoding the C-terminal part of **urokinase** (from residue 139 to the end), was constructed. **Recombinant** fusion protein produced by COS1 or R1610 cells transfected with this DNA had a specific activity of 73,000 units/mg.

L98 ANSWER 71 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
 AN 1988:628605 HCAPLUS  
 DN 109:228605  
 TI Yeast secretion of mammalian proteins: a comparison of  
**prourokinase**, prochymosin and alpha-1-antitrypsin  
 AU Moir, Donald T.  
 CS Collab. Res., Inc., Bedford, MA, 01730, USA  
 SO World Biotech Rep. (1987), Volume 1, Issue Pt. 3, 39-45  
 Publisher: Online Publ., London, UK.  
 CODEN: 56IUA3

DT Conference

LA English

AB Mammalian proteins secreted from bakers' yeast are modified post-translationally in two important ways: disulfide bonds form properly and the correct asparagines are glycosylated. The secretion signal, host strain, mode of maintenance in the cell, and the particular mammalian gene all influence the efficiency of secretion from yeast. Calf prochymosin and human **prourokinase** secreted from yeast, in contrast to those proteins produced intracellularly in **Escherichia coli** or yeast, are properly folded and exhibit full specific activity. Human alpha-1-anti-trypsin secreted from yeast is glycosylated at all three asparagines, and yeast **mutants** alter the pattern of the carbohydrate applied.

IT 82657-92-9P, **Prourokinase**

RL: PREP (Preparation)

(manufacture of human, by **recombinant** yeast)

L98 ANSWER 72 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
 AN 1988:523794 HCAPLUS  
 DN 109:123794

TI Human **prourokinase mutants**, method for producing the same, DNA sequences encoding them, plasmids containing the same, and transformants containing them

IN Kasai, Shunji; Hiramatsu, Ryuji; Uno, Shusei; Nagai, Masanori; Arimura, Hirofumi

PA Green Cross Corp., Japan

SO Eur. Pat. Appl., 41 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 253241	A1	19880120	EP 1987-109628	19870703 <--
	R: BE, CH, DE, ES, FR, GB, LI, NL, SE				
	JP 63146789	A2	19880618	JP 1987-36495	19870218 <--
	JP 07061266	B4	19950705		
	US 5098840	A	19920324	US 1990-525011	19900518 <--
PRAI	JP 1986-156936	A	19860703	<--	
	JP 1987-36495	A	19870218	<--	
	US 1987-70003	B2	19870706	<--	
	JP 1989-126433	A	19890518	<--	
	JP 1989-126434	A	19890518	<--	
	US 1989-433938	B2	19891109	<--	
	JP 1990-42020	A	19900222	<--	

AB A human **prourokinase mutant** (I) having longer half-life in blood and less bleeding side-effects is manufactured using genetic engineering techniques. Some or all of the epidermal growth factor (EGF) domain has been deleted or replaced by one or more different amino acid residues. The synthetic oligonucleotide encoding the **mutant I** lacking the Asn10-Asp45 region was prepared and used in constructing the expression plasmid pSV-UK11. CHO-K1 cells transformed with pSV-UK11

produced I having a half-life in blood of 6.25 min and similar activity to the natural human urine **urokinase**.

IT 116284-13-0, Deoxyribonucleic acid (human clone pUK4/pUK18 (1-9)-(43-411) **prourokinase**-specifying) 116284-14-1, Deoxyribonucleic acid (human clone pUK4/pUK18 (1-9)-(46-411)-**prourokinase**-specifying) 116284-15-2, Deoxyribonucleic acid (human clone pUK4/pUK18 (1-9)-(50-411)-**prourokinase**-specifying)

RL: PRP (Properties)

(expression in CHO cells and nucleotide sequence of)

L98 ANSWER 73 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1988:433212 HCAPLUS

DN 109:33212

TI Construction of **Escherichia coli** expression vectors encoding hybrid tissue plasminogen activator-**urokinase** proteins, and preparation of said proteins

IN Tagawa, Michito; Wada, Masakatsu; Yamada, Masayuki; Yokoyama, Midori; Numao, Naganori

PA Sagami Chemical Research Center, Japan; Central Glass Co., Ltd.; Hodogaya Chemical Co., Ltd.; Nippon Soda Co., Ltd.; Nissan Chemical Industries, Ltd.; Toyo Soda Mfg. Co., Ltd.

SO Eur. Pat. Appl., 65 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 231883	A1	19870812	EP 1987-101209	19870129 <--
	EP 231883	B1	19920902		
	R: BE, CH, DE, ES, FR, GB, IT, LI, NL, SE				
	ES 2043610	T3	19940101	ES 1987-101209	19870129 <--
	DK 8700509	A	19870801	DK 1987-509	19870130 <--
	DK 175604	B1	20041220		
	WO 8704720	A1	19870813	WO 1987-JP65	19870130 <--
	W: SU				
	JP 62272975	A2	19871127	JP 1987-18626	19870130 <--
	JP 2589687	B2	19970312		
	SU 1732814	A3	19920507	SU 1987-4028997	19870130 <--
	US 5204255	A	19930420	US 1991-726129	19910703 <--
PRAI	JP 1986-17734	A	19860131	<--	
	US 1987-7865	B1	19870128	<--	

AB Hybrid proteins containing N-terminal fibrin binding domain(s) from tissue plasminogen activator (tPA) and a C-terminal **prourokinase** (PK) polypeptide having plasminogen activator activity are produced with **E. coli** transformed with expression vectors encoding these proteins. Plasmid pHA03, encoding a hybrid protein consisting of residues 161-219 of tPA at the N-terminal and residues 150-411 of PK in which Phe-157 is changed to aspartic acid (to inhibit proteolytic degradation) at the C-terminal, was constructed. Expression of the protein was under the control of the tac promoter and the C230 Shine-Dalgarno sequence. This hybrid protein, expressed in **E. coli**, had the same affinity for a fibrin affinity column as did tPA; the same enzymic activity as PK, i.e. a  $K_m = 2.0 \times 10^{-4}$  mol/L (using synthetic substrate S2288); and an increased resistance to proteolytic inactivation by plasmin and thrombin.

IT 115283-71-1 115283-72-2 115283-73-3  
115283-75-5 115283-76-6 115283-77-7  
115283-78-8 115283-79-9 115283-80-2  
115283-81-3

RL: PRP (Properties); BIOL (Biological study)

(amino acid sequence of and cloning and expression in

- Escherichia coli** of cDNA for)
- IT 115283-74-4  
RL: PRP (Properties)  
(amino acid sequence of and cloning and expression in **Escherichia coli** of cDNA of)
- IT 82657-92-9D, **Prourokinase**, fusion products  
RL: PRP (Properties)  
(enzymic region of, with fibrin binding region of tissue-type plasminogen activator, manufacture in **Escherichia coli** of)
- IT 115283-41-5 115283-42-6 115283-43-7  
115283-44-8 115283-45-9 115283-46-0  
115283-47-1 115283-48-2 115283-49-3  
115283-50-6 115283-51-7  
RL: PRP (Properties); BIOL (Biological study)  
(nucleotide sequence and cloning and expression in **Escherichia coli** of)
- L98 ANSWER 74 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 1988:162640 HCAPLUS  
DN 108:162640  
TI Enhanced expression of human **pro-urokinase** cDNA in **Escherichia coli**  
AU Hibino, Yasuo; Miyake, Toshio; Kobayashi, Yohichi; Ohmori, Muneki; Miki, Tetsuzo; Matsumoto, Reiko; Numao, Naganori; Kondo, Kiyosi  
CS Sagami Chem. Res. Cent., Kanagawa, 229, Japan  
SO Agricultural and Biological Chemistry (1988), 52(2), 329-36  
CODEN: ABCHA6; ISSN: 0002-1369  
DT Journal  
LA English  
AB Human **pro-urokinase** cDNA was isolated from the cDNA library constructed from human kidney mRNA using the dC/dG homopolymer tailing method and Okayama-Berg method with pBR322 as a vector. A mature polypeptide starting with Ser was produced in **E. coli** under the control of the tac promoter and the **Shine-Dalgarno** sequence of the catechol 2,3-oxygenase gene derived from *Pseudomonas putida*. By replacing the sequence coding for N-terminal eight amino acids of **pro-urokinase** with the synthetic DNA oligomer, the bacterial **pro-urokinase** had a mol. weight of 47,000 daltons and accounted for 15% of the insol. fraction of **E. coli** proteins in induced cells. Its biol. activity was restored by renaturing the bacterial product. The activity of bacterial **pro-urokinase** was 450 IU/mL culture.
- IT 82657-92-9, **Pro-urokinase**  
RL: PRP (Properties)  
(cDNA for, of human, cloning and expression of)
- L98 ANSWER 75 OF 75 HCAPLUS COPYRIGHT 2005 ACS on STN  
AN 1985:434019 HCAPLUS  
DN 103:34019  
TI Characterization of the high-affinity interaction between human plasminogen and **pro-urokinase**  
AU Lijnen, H. Roger; Collen, Desire  
CS Cent. Thromb. Vasc. Res., Univ. Leuven, Louvain, Belg.  
SO European Journal of Biochemistry (1985), 150(1), 141-4  
CODEN: EJBCAI; ISSN: 0014-2956  
DT Journal  
LA English  
AB Activation of human plasminogen (I), a modified plasminogen with N-terminal **lysine**, valine, or methionine (II), and low-mol.-weight plasminogen [lacking **lysine**-binding sites (LBSs)] (III) by **prourokinase (pro-UK)**, obtained from a human lung adenocarcinoma cell line (Calu-3, ATCC), obeys Michaelis-Menten

kinetics. Activation occurs with a comparable affinity ( $K_m$ , 0.40-0.77  $\mu M$ ), whereas the catalytic rate constant ( $k_{cat}$ ) is comparable for I (0.0022  $s^{-1}$ ) and III (0.0034  $s^{-1}$ ), but is somewhat higher for II (0.0106  $s^{-1}$ ). The rate of activation of I by **pro-UK** is not significantly influenced by the presence of 6-aminohexanoic acid, purified fragments LBS I or LBS II, or **histidine**-rich glycoprotein, indicating that the high affinity of **pro-UK** for I is not mediated via the high-affinity LBS I of I located in kringles 1-3 (LBS I) nor via the low-affinity LBS II within kringle 4 (LBS II). Apparently the site(s) in I involved in the high-affinity interaction with **pro-UK** are located within the III moiety.

IT 82657-92-9

RL: BIOL (Biological study)

(plasminogen of human activation by, of lung adenocarcinoma cells, kinetics of)

=> => fil biosis

FILE 'BIOSIS' ENTERED AT 07:32:13 ON 21 APR 2005

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FILE COVERS 1969 TO DATE.

CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNS) PRESENT FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 20 April 2005 (20050420/ED)

FILE RELOADED: 19 October 2003.

=> d all tot

L106 ANSWER 1 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
AN 1999:111090 BIOSIS  
DN PREV199900111090  
TI Production of human **prourokinase**.  
AU Brandazza, A. [Inventor]; **Sarmientos, P.** [Inventor]; Orsini, G. [Inventor]  
CS Rivolta d'Adda, Italy  
ASSIGNEE: VASCULAR LABORATORY INC.  
PI US 5866358 Feb. 2, 1999  
SO Official Gazette of the United States Patent and Trademark Office Patents, (Feb. 2, 1999) Vol. 1219, No. 1, pp. 488. print.  
CODEN: OGUPE7. ISSN: 0098-1133.  
DT **Patent**  
LA English  
ED Entered STN: 12 Mar 1999  
Last Updated on STN: 12 Mar 1999  
NCL 435069100  
CC General biology - Miscellaneous 00532  
IT Major Concepts  
Biochemistry and Molecular Biophysics; Bioprocess Engineering; Enzymology (Biochemistry and Molecular Biophysics); Infection; Methods and Techniques  
IT Miscellaneous Descriptors  
BACTERIA; BIOTECHNOLOGY; HUMAN **PROUROKINASE** PRODUCTION; METHODS  
ORGN Classifier  
Enterobacteriaceae 06702  
Super Taxa  
Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria; Microorganisms  
Organism Name  
Escherichia coli

## Taxa Notes

Bacteria, Eubacteria, Microorganisms

## ORGN Classifier

Microorganisms 01000

## Super Taxa

Microorganisms

## Organism Name

microorganism

## Taxa Notes

Microorganisms

L106 ANSWER 2 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

AN 1997:17614 BIOSIS

DN PREV199799316817

TI A site-directed mutagenesis of **pro-urokinase** which substantially reduces its intrinsic activity.AU Liu, Jian-Ning [Reprint author]; Tang, Wei; Sun, Zi-Yong; Kung, Wendy; Pannell, Ralph; **Sarmientos, Paolo**; Gurewich, Victor

CS Vasc. Res. Laboratory, Burlington Build., Room 554B, Deaconess Hosp., One Deaconess Rd., Boston, MA 02215, USA

SO Biochemistry, (1996) Vol. 35, No. 45, pp. 14070-14076.

CODEN: BICHAW. ISSN: 0006-2960.

DT Article

LA English

ED Entered STN: 15 Jan 1997

Last Updated on STN: 23 Jan 1997

AB **Single-chain urokinase**-type plasminogen

activator or **pro-urokinase** is a zymogen with an intrinsic catalytic activity which is greater than that of most other zymogens. To study the structural basis for this activity, a three-dimensional homology model was calculated using the crystallographic structure of chymotrypsinogen, and the structure-function relationship was studied using site-directed mutagenesis and kinetic analysis. This model revealed a unique Lys-300 in **pro-urokinase** which could form a weak interaction with Asp-355, adjacent to the active site Ser-356. It was postulated that this lysine, by its epsilon-amino group, may serve to pull Ser-356 close to the active position, thereby inducing the higher intrinsic activity of **pro-urokinase**. This was consistent with the published finding that a homologous lysine (Lys-416) in single chain tissue plasminogen activator when mutated to serine induced some reduction in activity. To test this hypothesis, a site-directed mutant with a neutral residue (Lys-300 to fdaarw Ala) was produced and characterized. The Ala-300-**pro-urokinase** had a 40-fold lower amidolytic activity than that of **pro-urokinase**. It was also stable in plasma at much higher concentrations than **pro-urokinase**, reflecting much attenuated plasminogen activation. Plasmin activatability was comparable to that of **pro-urokinase**, but the resultant two-chain derivative (Ala-300-**urokinase**) had a lower enzymatic activity (approx 33% that of **urokinase**) due to a reduction of k-cat. Interestingly, the K-M of two-chain Ala-300-**urokinase** against plasminogen was 5.8-fold lower than that of **urokinase**, being similar to that of **pro-urokinase** which has a K-M about 5-fold lower than **urokinase**. In conclusion, the hypothesis that Lys-300 is a key structural determinant of the high intrinsic activity of **pro-urokinase** was confirmed by these studies. This residue also appears to be important for the full expression of the enzymatic activity of **urokinase**.

CC Biochemistry studies - Proteins, peptides and amino acids 10064

Biophysics - Molecular properties and macromolecules 10506

Enzymes - Chemical and physical 10806

Enzymes - Physiological studies 10808

IT Major Concepts

Biochemistry and Molecular Biophysics; Enzymology (Biochemistry and Molecular Biophysics)

IT Chemicals & Biochemicals  
**PRO-UROKINASE**; SERINE PROTEASE

IT Miscellaneous Descriptors  
 ANALYTICAL METHOD; COMPARISON; ENZYMOLOGY; **PRO-UROKINASE**; SERINE PROTEASE COMPARISON; SITE-DIRECTED  
 MUTAGENESIS; STRUCTURE-ACTIVITY RELATIONSHIP; THREE-DIMENSIONAL  
 STRUCTURE; ZYMOGEN

RN **82657-92-9 (PRO-UROKINASE)**  
 37259-58-8 (SERINE PROTEASE)

L106 ANSWER 3 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
 AN 1993:432624 BIOSIS  
 DN PREV199396087249  
 TI Implication of cystein residues in the activity of **single-chain urokinase-plasminogen activator**.  
 AU Hamelin, Jocelyne [Reprint author]; **Sarmientos, Paolo**; Orsini, Gaetano; Galibert, Francis  
 CS Lab. de Recombinaisons Genetiques, Centre Hayem, Hopital Saint-Louis, 75475, Paris Cedex 10, France  
 SO Biochemical and Biophysical Research Communications, (1993) Vol. 194, No. 2, pp. 978-985.  
 CODEN: BBRCA9. ISSN: 0006-291X.  
 DT Article  
 LA English  
 ED Entered STN: 22 Sep 1993  
 Last Updated on STN: 23 Sep 1993

AB **Single-chain urokinase-plasminogen activator** contains 24 cysteine residues involved in 12 disulfide bonds and distributed all along the three domains of the protein. In order to investigate the role of these disulfide bridges in the catalytic activities of **scu-PA**, we used site-specific mutagenesis to construct 10 mutants in which some cysteine residues were changed to serine residues. Each mutated DNA fragment was cloned into a procaryotic expression vector and the protein expressed in E. coli. Mutant proteins of the expected size were produced and analyzed for amidolytic and fibrinolytic activities. From this, it is shown that: i) the disulfide bonds in the epidermal growth factor (EGF)-like and in the kringle domains are not necessary. Moreover, disulfide bond deletion in the kringle domain improves those catalytic activities; ii) on the contrary, the disulfide bridges in the catalytic domain are essential for maintaining both activities.

CC Genetics - General 03502  
 Biochemistry studies - Nucleic acids, purines and pyrimidines 10062  
 Biochemistry studies - Proteins, peptides and amino acids 10064  
 Biophysics - Methods and techniques 10504  
 Biophysics - Molecular properties and macromolecules 10506  
 Enzymes - Chemical and physical 10806  
 Enzymes - Physiological studies 10808  
 Metabolism - Nucleic acids, purines and pyrimidines 13014  
 Blood - Blood and lymph studies 15002

IT Major Concepts  
 Biochemistry and Molecular Biophysics; Enzymology (Biochemistry and Molecular Biophysics); Genetics; Metabolism; Methods and Techniques

IT Chemicals & Biochemicals  
 CYSTEIN

IT Miscellaneous Descriptors  
 CARCINOGENESIS; DEVELOPMENTAL REGULATION; HUMAN GASTRIC CANCER  
 COMPLEMENTARY DNA; NORTHERN BLOT ANALYSIS; RAT EMBRYO STOMACH

ORGN Classifier  
 Animalia 33000  
 Super Taxa

Animalia  
 Organism Name  
 Animalia  
 Taxa Notes  
 Animals  
 RN 52-90-4 (CYSTEIN)

L106 ANSWER 4 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
 AN 1992:408842 BIOSIS  
 DN PREV199294072042; BA94:72042  
 TI STRUCTURE-FUNCTION RELATIONSHIP OF BASIC FIBROBLAST GROWTH FACTOR  
 SITE-DIRECTED MUTAGENESIS OF A PUTATIVE HEPARIN-BINDING AND  
 RECEPTOR-REGION.  
 AU PRESTA M [Reprint author]; STATUTO M; ISACCHI A; CACCIA P; POZZI A;  
 GUALANDRIS A; RUSNATI M; BERGONZONI L; **SARMIENTOS P**  
 CS UNIT GENERAL PATHOL AND IMMUNOL, DEP BIOMEDICAL SCIENCES AND BIOTECHNOL,  
 SCH MED, UNIV BRESCIA, 25123 BRESCIA, ITALY  
 SO Biochemical and Biophysical Research Communications, (1992) Vol. 185, No.  
 3, pp. 1098-1107.  
 CODEN: BBRCA9. ISSN: 0006-291X.  
 DT Article  
 FS BA  
 LA ENGLISH  
 ED Entered STN: 9 Sep 1992  
 Last Updated on STN: 9 Sep 1992  
 AB Basic residues Arg-118, Lys-119, Lys-128, and Arg-129 within a putative  
 heparin-binding and receptor-binding region of the 155-amino acid form of  
 basic fibroblast growth factor (bFGF) have been changed to neutral  
 glutamine residues by site-directed mutagenesis of the human bFGF cDNA.  
 The bFGF mutant (M6B-bFGF) was expressed in E. coli and purified to  
 homogeneity. When compared to wild type bFGF, M6B-bFGF showed in cultured  
 endothelial cells a similar receptor-binding capacity and mitogenic  
 activity, but a reduced affinity for heparin-like low affinity binding  
 sites, a reduced chemotactic activity, and a reduced capacity to induce  
 the production of urokinase-type plasminogen activator. In  
 vivo, M6B-bFGF lacked a significant angiogenic activity. Modifications of  
 both the primary and the tertiary structure of bFGF appear to be  
 responsible for the modified biological properties of M6B-bFGF, thus  
 confirming the possibility to dissociate at the structural level some of  
 the biological activities exerted by bFGF on endothelial cells.  
 CC Cytology - Human 02508  
 Biochemistry studies - Nucleic acids, purines and pyrimidines 10062  
 Biochemistry studies - Proteins, peptides and amino acids 10064  
 Biochemistry studies - Carbohydrates 10068  
 Cardiovascular system - Physiology and biochemistry 14504  
 Endocrine - General 17002  
 Physiology and biochemistry of bacteria 31000  
 IT Major Concepts  
 Biochemistry and Molecular Biophysics; Cardiovascular System (Transport  
 and Circulation); Cell Biology; Endocrine System (Chemical Coordination  
 and Homeostasis); Physiology  
 IT Miscellaneous Descriptors  
 ESCHERICHIA-COLI HUMAN ENDOTHELIAL CELLS ARGININE-118 LYSINE-119  
 LYSINE-128 ARGININE-129 COMPLEMENTARY DNA  
 ORGN Classifier  
 Enterobacteriaceae 06702  
 Super Taxa  
 Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria;  
 Microorganisms  
 Taxa Notes  
 Bacteria, Eubacteria, Microorganisms  
 ORGN Classifier  
 Hominidae 86215

## Super Taxa

Primates; Mammalia; Vertebrata; Chordata; Animalia

## Taxa Notes

Animals, Chordates, Humans, Mammals, Primates, Vertebrates

L106 ANSWER 5 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

AN 1991:501012 BIOSIS

DN PREV199192123972; BA92:123972

TI USE OF THE **UROKINASE**-TYPE PLASMINOGEN ACTIVATOR GENE AS A  
 GENERAL TOOL TO MONITOR EXPRESSION IN TRANSGENIC ANIMALS STUDY OF THE  
 TISSUE-SPECIFICITY OF THE MURINE WHEY ACIDIC PROTEIN WAP EXPRESSION  
 SIGNALS.

AU BRANDAZZA A [Reprint author]; LEE E; FERRERA M; TILLMAN U; **SARMIENTOS**  
 P; WESTPHAL H

CS FARMITALIA, CARLO ERBA, DEP BIOTECHNOLOGY, VIALE BEZZI 24, 20146 MILANO,  
 ITALY

SO Journal of Biotechnology, (1991) Vol. 20, No. 2, pp. 201-212.

CODEN: JBITD4. ISSN: 0168-1656.

DT Article

FS BA

LA ENGLISH

ED Entered STN: 12 Nov 1991

Last Updated on STN: 13 Nov 1991

AB **Urokinase**-type plasminogen activator (uPA) is a proteolytic  
 enzyme able to convert the zymogen plasminogen into the strong protease  
 plasmin. The availability of very sensitive tests to measure the  
 enzymatic activity of a plasminogen activator renders the corresponding  
 gene an ideal candidate for the detection of promoter activity. In this  
 paper we describe the utilization of the human uPA gene as detector of  
 tissue-specificity of the murine whey acidic protein (WAP) expression  
 signals in transgenic mice. The WAP promoter has been previously  
 investigated for the production of foreign proteins in the milk of  
 transgenic animals. In our genetic constructions, the human uPA cDNA was  
 linked to the promoter region as well as to 3'-end distal sequences of the  
 WAP gene. Five transgenic lines were obtained in which, however,  
 expression levels of human uPA in the milk were still quite low.  
 Surprisingly, four of these five positive transgenic mice show a  
 consistent activity of the WAP promoter in brain extracts compared to  
 other tissues.

CC Genetics - General 03502

Genetics - Animal 03506

Genetics - Human 03508

Comparative biochemistry 10010

Biochemistry methods - General 10050

Biochemistry methods - Nucleic acids, purines and pyrimidines 10052

Biochemistry methods - Proteins, peptides and amino acids 10054

Biochemistry studies - General 10060

Biochemistry studies - Nucleic acids, purines and pyrimidines 10062

Biochemistry studies - Proteins, peptides and amino acids 10064

Biophysics - Molecular properties and macromolecules 10506

Enzymes - Methods 10804

Enzymes - Chemical and physical 10806

Enzymes - Physiological studies 10808

Physiology - General 12002

Metabolism - General metabolism and metabolic pathways 13002

Metabolism - Proteins, peptides and amino acids 13012

Metabolism - Nucleic acids, purines and pyrimidines 13014

Blood - General and methods 15001

Blood - Blood and lymph studies 15002

Reproductive system - Physiology and biochemistry 16504

Nervous system - Physiology and biochemistry 20504

Laboratory animals - General 28002

IT Major Concepts

Animal Care; Biochemistry and Molecular Biophysics; Blood and Lymphatics (Transport and Circulation); Enzymology (Biochemistry and Molecular Biophysics); Genetics; Metabolism; Nervous System (Neural Coordination); Reproductive System (Reproduction)

IT Miscellaneous Descriptors

HUMAN GENE COMPARISON ENZYMATIC ACTIVITY MILK PROMOTER METHOD GENETIC ENGINEERING

ORGN Classifier

Hominidae 86215

Super Taxa

Primates; Mammalia; Vertebrata; Chordata; Animalia

Taxa Notes

Animals, Chordates, Humans, Mammals, Primates, Vertebrates

ORGN Classifier

Muridae 86375

Super Taxa

Rodentia; Mammalia; Vertebrata; Chordata; Animalia

Taxa Notes

Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

RN 9039-53-6 (UROKINASE)

L106 ANSWER 6 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

AN 1991:248288 BIOSIS

DN PREV199191128843; BA91:128843

TI A SIX-AMINO ACID DELETION IN BASIC FIBROBLAST GROWTH FACTOR DISSOCIATES ITS MITOGENIC ACTIVITY FROM ITS PLASMINOGEN ACTIVATOR-INDUCING CAPACITY.

AU ISACCHI A [Reprint author]; STATUTO M; CHIESA R; BERGONZONI L; RUSNATI M; **SARMIENTOS P**; RAGNOTTI G; PRESTA M

CS GENERAL PATHOL, DEP BIOMEDICAL SCI, SCH MED, UNIV BRESCIA, VIA VALSABBINA 19, 25133 BRESCIA, ITALY

SO Proceedings of the National Academy of Sciences of the United States of America, (1991) Vol. 88, No. 7, pp. 2628-2632.

CODEN: PNASA6. ISSN: 0027-8424.

DT Article

FS BA

LA ENGLISH

ED Entered STN: 25 May 1991

Last Updated on STN: 25 May 1991

AB A recombinant deletion mutant of the 155-amino acid form of human basic fibroblast growth factor (bFGF), lacking amino acid residues 27-32 (Lys-Asp-Pro-Lys-Arg-Leu), was expressed in *Escherichia coli* and purified to homogeneity by heparin-Sepharose affinity chromatography. When maintained in the presence of an equimolar concentration of soluble heparin, the bFGF mutant (M1-bFGF) is as potent as bFGF in stimulating cell proliferation in normal and transformed fetal bovine aortic endothelial cells, in adult bovine aortic endothelial cells, and in NIH 3T3 fibroblasts. However, under the same experimental conditions, M1-bFGF is at least 100 times less efficient than bFGF in stimulating plasminogen activator (PA) production in endothelial cells, as assayed by chromogenic PA assay, SDS/PAGE zymography, and Northern blot analysis of **urokinase**-type PA mRNA. In the presence of heparin, M1-bFGF binds to bFGF plasma membrane receptors present on endothelial cells in a manner undistinguishable from bFGF. It also induces the same tyrosine phosphorylation pattern when added to NIH 3T3 cells. The data suggest that the PA-inducing activity of bFGF may depend upon a functional domain that differs from those involved in the mitogenic activity of the growth factor and that the binding of bFGF to its plasma membrane receptor may not be sufficient to induce **urokinase**-type PA production in endothelial cells.

CC Cytology - Animal 02506

Biochemistry studies - Proteins, peptides and amino acids 10064

Biochemistry studies - Carbohydrates 10068

Biophysics - Molecular properties and macromolecules 10506  
 Cardiovascular system - Physiology and biochemistry 14504  
 Endocrine - General 17002

## IT Major Concepts

Biochemistry and Molecular Biophysics; Cardiovascular System (Transport and Circulation); Cell Biology; Endocrine System (Chemical Coordination and Homeostasis)

## IT Miscellaneous Descriptors

HUMAN 3T3 CELL BOVINE ENDOTHELIAL CELL RECEPTOR STRUCTURE FUNCTION

## ORGN Classifier

Bovidae 85715

## Super Taxa

Artiodactyla; Mammalia; Vertebrata; Chordata; Animalia

## Taxa Notes

Animals, Artiodactyls, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Vertebrates

## ORGN Classifier

Hominidae 86215

## Super Taxa

Primates; Mammalia; Vertebrata; Chordata; Animalia

## Taxa Notes

Animals, Chordates, Humans, Mammals, Primates, Vertebrates

## ORGN Classifier

Muridae 86375

## Super Taxa

Rodentia; Mammalia; Vertebrata; Chordata; Animalia

## Taxa Notes

Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

L106 ANSWER 7 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

AN 1991:230709 BIOSIS

DN PREV199191122169; BA91:122169

TI EFFICIENT RENATURATION AND FIBRINOLYTIC PROPERTIES OF **PROUROKINASE** AND A DELETION MUTANT EXPRESSED IN ESCHERICHIA-COLI AS INCLUSION BODIES.

AU ORSINI G [Reprint author]; BRANDAZZA A; **SARMIENTOS P**; MOLINARI A; LANSEN J; CAUET G

CS FARMITALIA, DEP BIOTECHNOL, VIALE BEZZI 24, I-20146 MILANO, ITALY

SO European Journal of Biochemistry, (1991) Vol. 195, No. 3, pp. 691-698.

CODEN: EJBCAI. ISSN: 0014-2956.

DT Article

FS BA

LA ENGLISH

ED Entered STN: 9 May 1991

Last Updated on STN: 10 May 1991

AB **Prourokinase** is a plasminogen activator of 411 amino acids which displays a clot-lysis activity through a fibrin-dependent mechanism, and which seems to be a promising agent for the treatment of acute myocardial infarction. The preparation of recombinant **prourokinase** in bacteria has been hampered by its insolubility and by difficulty in refolding in the polypeptide chain. In this paper we describe the renaturation process of two recombinant proteins expressed in *Escherichia coli* as inclusion bodies: **prourokinase** and a deletion derivative  $\Delta 125$ - **prourokinase** and 0.25 h<sup>-1</sup> for **prourokinase**). Our process involves sequential steps of denaturation, reduction and controlled refolding of the polypeptide chain. When applied to pure, non-glycosylated and active **prourokinase**, it gives a refolding yield of about 80%, demonstrating the efficiency of the renaturation procedure. Lower yields (15% and 30%), respectively, for **prourokinase** and  $\Delta 125$ - **prourokinase** were obtained when the same refolding protocol was applied to inclusion bodies from bacteria. After purification to homogeneity (as shown by HPLC and SDS/PAGE) specific activities were 160,000 and 250,000 IU/mg protein,

respectively, for **prourokinase** and  $\Delta 125$ -**prourokinase**. As with **prourokinase**, the deletion mutant  $\Delta 125$ - **prourokinase** displays a zymogenic nature, being activation by plasmin to the active two-chain form; however, this mutant is approximately fourfold more resistant than **prourokinase** to plasmin activation, and consequently shows a different fibrinolytic profile.

CC Biochemistry studies - Proteins, peptides and amino acids 10064  
 Enzymes - Chemical and physical 10806  
 Blood - Blood and lymph studies 15002  
 Pharmacology - Blood and hematopoietic agents 22008  
 Physiology and biochemistry of bacteria 31000  
 Genetics of bacteria and viruses 31500  
 Food microbiology - Antibiotics, biologics and other agents 39004  
 IT Major Concepts  
   Biochemistry and Molecular Biophysics; Blood and Lymphatics (Transport and Circulation); Enzymology (Biochemistry and Molecular Biophysics); Pharmacology  
 IT Miscellaneous Descriptors  
   RECOMBINANT PROTEINS GENETICALLY-ENGINEERED PROTEINS THROMBOLYTIC AGENTS PROTEIN FOLDING  
 ORGN Classifier  
   Enterobacteriaceae 06702  
   Super Taxa  
     Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria; Microorganisms  
   Taxa Notes  
     Bacteria, Eubacteria, Microorganisms  
 RN 82657-92-9 (PROUROKINASE)

=> d his

(FILE 'HOME' ENTERED AT 06:51:51 ON 21 APR 2005)  
 DEL HIS

FILE 'HCAPLUS' ENTERED AT 06:53:57 ON 21 APR 2005

L1 712 S ?PROUK? OR ?PROUROKINASE? OR PRO() (UK OR UROKINASE)  
 L2 3 S RPRO() (UK OR UROKINASE)  
 L3 712 S L1,L2

FILE 'REGISTRY' ENTERED AT 06:55:45 ON 21 APR 2005

L4 1 S 82657-92-9  
   E PROUROKINASE  
 L5 151 S E3

FILE 'HCAPLUS' ENTERED AT 06:57:31 ON 21 APR 2005

L6 684 S L4  
 L7 714 S L5  
 L8 545 S ABT 187 OR ABT187 OR PRO U PA OR PROLYSE OR PROLYZE OR PUK OR  
 L9 1171 S L3,L6-L8  
 L10 7 S PET29A  
 L11 20 S ?PET29?  
 L12 4 S L9 AND L10,L11  
 L13 9 S L9 AND T7  
 L14 6 S L9 AND (SHINE OR DALGARNO)  
 L15 16 S L9 AND (BL21 OR DE3 OR RIL)  
 L16 7 S L12-L14 AND L15  
   E E COLI/CT  
   E ESCHERICHIA/CT  
 L17 148991 S E3+OLD,NT,PFT,RT OR E14+OLD,NT,PFT,RT  
 L18 248421 S ("E" OR ESCHERICH?) ()COLI  
 L19 176 S L9 AND L17,L18

L20 2 S L19 AND TYPE B  
L21 108 S L9 AND B  
L22 3 S L20,L21 AND L10-L16  
L23 153 S L9 AND ESCHERI?  
L24 21 S L23 AND L10-L16  
L25 3 S L24 AND (TYPE B OR B)  
L26 27 S L12-L16,L20,L22,L25  
L27 235 S L19,L21,L23,L24 NOT L26  
L28 4 S L26 AND ?MUTANT?  
L29 2 S L26 AND ?MUTAT?  
L30 36 S L27 AND (?MUTANT? OR MUTAT?)  
L31 2 S L26 AND ?MUTAGEN?  
L32 15 S L27 AND ?MUTAGEN?  
L33 4 S L28,L29,L31  
L34 41 S L30,L32 NOT L33  
L35 2 S L9 AND FLEX? (L) LOOP?  
L36 1 S L9 AND WOBBL? (L) LOOP?  
L37 2 S L35,L36  
L38 6 S L9 AND (LYS300 OR LYS 300)  
L39 0 S L9 AND (LYSINE300 OR LYSINE 300)  
L40 27 S L9 AND (HIS OR HISTID?)  
L41 124 S L9 AND (LYS OR LYSINE OR LYSYL?)  
L42 12 S L40 AND L41

FILE 'REGISTRY' ENTERED AT 07:06:40 ON 21 APR 2005

L43 1 S 56-87-1  
L44 3 S (D-LYSINE OR DL-LYSINE)/CN OR L43  
L45 3 S (L-HISTIDINE OR D-HISTIDINE OR DL-HISTIDINE)/CN

FILE 'HCAPLUS' ENTERED AT 07:07:20 ON 21 APR 2005

L46 24 S L44 AND L9  
L47 6 S L45 AND L9  
L48 13 S L38,L41,L46 AND L40,L47  
L49 2 S L48 AND L10-L16,L26  
L50 4 S L48 AND L27  
L51 3 S L48 AND L28-L37  
L52 6 S L49-L51  
L53 5 S L52 NOT CRYOGEL/TI  
L54 96 S L10-L16,L20,L22,L25,L26,L28-L37,L48  
L55 5 S L54 AND L53  
L56 4 S L55 NOT MMP 3/TI  
L57 91 S L54 NOT L55  
L58 15 S L57 AND PROUROKINASE/TI  
L59 19 S L56,L58  
L60 76 S L57 NOT L59  
L61 35 S L60 AND L6  
L62 38 S L60 AND L7  
L63 57 S L61,L62,L59  
L64 38 S L54 NOT L55-L56,L58,L59,L61-L63  
SEL DN AN 13-16 22 24 26 29-34 36-38  
L65 16 S L64 AND E1-E48  
L66 73 S L63,L65  
L67 72 S L66 AND ?UROKINASE?  
L68 1 S L66 NOT L67  
E SARMIENTOS P/AU  
L69 46 S E3,E4  
E PAGANI M/AU  
L70 45 S E3-E8,E18  
L71 9 S L69,L70 AND L9  
L72 12 S L69,L70 AND ?UROKINASE?  
L73 12 S L71,L72  
L74 2 S US20050019863/PN OR (WO2004-US11840 OR US2004-826598# OR US20  
L75 1 S L74 AND L9

L76 1 S L74 AND ?UROKINASE?  
 L77 12 S L75,L76,L73  
 L78 78 S L77,L67  
 L79 78 S L78 AND L1-L3,L6-L42,L46-L78  
 L80 75 S L79 AND (PD<=20030418 OR PRD<=20030418 OR AD<=20030418)  
 L81 3 S L79 NOT L80  
 L82 78 S L79-L80  
 L83 49 S L82 AND (?MUTANT? OR ?MUTAGEN? OR ?MUTAT?)  
     E MUTANT/CT  
     E MUTAT/CT  
 L84 332100 S E7+OLD,NT,PFT,RT  
     E MUTAGEN/CT  
 L85 202397 S E5+OLD,NT,PFT,RT OR E5-E10  
 L86 268465 S E16+OLD,NT,PFT,RT  
 L87 13 S L82 AND L84-L86  
 L88 50 S L83,L87  
 L89 45 S L82 AND (RECOMBIN? OR CHIMER?)  
     E RECOMBINANT/CT  
 L90 0 S L82 AND E11+OLD,NT,PFT,RT  
 L91 0 S L82 AND E41+OLD,NT,PFT,RT  
 L92 28 S L82 AND E48+OLD,NT,PFT,RT  
     E E48+ALL  
 L93 28 S L82 AND E9+OLD,NT,PFT,RT  
 L94 4 S L82 AND E7+OLD,NT,PFT,RT  
 L95 70 S L88-L94  
 L96 8 S L82 NOT L95  
     SEL DN AN 1 4 5  
 L97 5 S L96 NOT E1-E9  
 L98 75 S L95,L97

FILE 'REGISTRY' ENTERED AT 07:29:12 ON 21 APR 2005

FILE 'HCAPLUS' ENTERED AT 07:29:23 ON 21 APR 2005

FILE 'BIOSIS' ENTERED AT 07:30:00 ON 21 APR 2005

    E SARMIENTOS P/AU  
 L99 39 S E3,E4  
     E PAGANI M/AU  
 L100 269 S E3-E11,E21  
 L101 1043 S L1,L2,L4,L5,L8  
 L102 12623 S ?UROKINASE?  
 L103 7 S L99,L100 AND L101,L102  
 L104 7 S L103 AND PY<=2003  
 L105 1 S L103 AND P/DT  
 L106 7 S L104,L105

FILE 'BIOSIS' ENTERED AT 07:32:13 ON 21 APR 2005

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